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ON THE TRANSMISSIBILITY BY *GLOSSINA* OF *TRYPANOSOMA BRUCEI*, *T. RHODESIENSE* AND *T. GAMBIENSE*, WITH SPECIAL REFERENCE TO OLD LABORATORY STRAINS

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MANY years ago Bouet and Roubaud (1910), working in Dahomey with strains of *Trypanosoma brucei*, *T. evansi* and *T. gambiense* brought from the Pasteur Institute in Paris, found that they could not transmit them by *Glossina*.

In 1912 Kleine and Fischer (1913), in East Africa, noted that some of their strains of *T. gambiense* were either non-transmissible cyclically or experienced very great difficulty in infecting tsetse. A year later Kleine, Fischer and Eckard (1914) decided that strains of trypanosomes which have recently passed cyclically through *Glossina* are better able to infect fly than strains that have been long in the mammal.

Reichenow (1921) was the first to report the phenomenon of non-transmissibility by *Glossina* strains of *T. gambiense* in man himself. He found that in flies fed on old cases of this variety of trypanosomiasis invasion of the salivary glands did not take place, and that infections even of the gut of the fly were rare. The number of flies employed in his experiments is not stated, but subsequent experience in Uganda makes it highly probable that some at least of Reichenow's strains were non-transmissible by tsetse, if not completely non-infective to the fly. That long residence of the trypanosome in one and the same host is not inevitably attended by permanent and complete loss of transmissibility has been sufficiently demonstrated in recent work from Entebbe. The transmissibility of a strain may indeed be greatly reduced or even completely in abeyance while it remains in the chronically infected host, but on removal of the trypanosome by direct inoculation into a new host it will often be found to have recovered this character (Duke, 1931). Then again, some strains isolated from human cases of long standing have been shown to be transmissible (Duke, 1930), whereas non-transmissible strains have been found in cases not obviously advanced. It must however be remembered that in chronic cases of trypanosomiasis in man it is not always possible to exclude a superinfection with another strain long after the first onset of the disease.

The work of Razgha (1929) and Reichenow (1932) on the culturability *in vitro* of various strains of *T. gambiense* and *T. rhodesiense* has shown that this character varies inversely with the length of time the strain has been removed from contact with *Glossina*; and Reichenow believes that the power to survive

and multiply in the culture tube and the power to infect tsetse are both manifestations of the same quality, which we may for convenience call transmissibility.

My own attention was first directed to the subject of non-transmissible strains in 1922, when a polymorphic trypanosome originally recovered in 1920 from wild *G. palpalis* on Lake Victoria was found to have lost the power of infecting the glands of the fly, and about the same time the same inability to invade the glands was detected in another strain which had originally been transmissible at the laboratory. Eventually in 1926, when the League of Nations Commission came to Uganda for some 18 months' study of the problems of human trypanosomiasis, opportunity was afforded for a more thorough investigation of this interesting subject. Thenceforth from time to time papers have been published from Entebbe about the transmissibility of trypanosomes, and there is now, in my own mind at any rate, no longer any doubt that in East and Central Africa loss of the power to pass cyclically through *Glossina* is a failing to which certain trypanosomes of the polymorphic group are very liable, although the conditions that determine the onset of this change are not yet fully understood.

T. brucei and *T. rhodesiense* appear to be much less susceptible to impairment of transmissibility than *T. gambiense*. It is possible that much depends on the species of mammal in which the trypanosome is constrained to spend its life. Evidence is steadily accumulating to show that the antelope of Africa are peculiarly suited to the three important polymorphic trypanosomes that depend on *Glossina* for their transmission in nature, viz. *T. brucei*, *T. gambiense* and *T. rhodesiense*. The ability of *T. gambiense* to survive for at least 22 months in an antelope was demonstrated years ago in Uganda (Duke, 1912), and in the last few months experiments (unpublished) at Entebbe have shown that a similar or even closer adjustment exists between antelope and *T. rhodesiense*.

In man and in domestic ruminants *T. gambiense* is clearly more susceptible to loss of transmissibility by tsetse than either *T. rhodesiense* or *T. brucei* (Duke, 1928); and strains of trypanosomes recently isolated from human beings naturally infected in Africa have been found to be not only non-transmissible by tsetse but almost if not completely non-infective to *Glossina*. I have restricted the above observations to East and Central Africa where my own experience has been acquired. Taylor and his colleagues in Nigeria have not yet satisfied themselves of the existence of such strains in West Africa, and it is clear from their published work that they do not expect to do so. As remarked elsewhere, the climate of Nigeria differs enormously from that of Uganda. The tsetse are different and so perhaps are the trypanosomes, for the history of human trypanosomiasis in Africa teaches that the west coast is the original home of the disease. It is possible that *T. gambiense* in West Africa has attained a degree of adjustment to man approaching that of *T. brucei* to game, and that the tendency to lose contact with *Glossina* has in consequence been reduced

or altogether eliminated. But the evidence hitherto adduced in favour of this assumption appears to me to be inadequate, and the range of adaptation to tsetse shown by Taylor's strains suggests that further investigation will show *T. gambiense*, east or west, to possess much the same characteristics.

The present paper is a further contribution to the study of the transmissibility of the polymorphic group of trypanosomes. It deals particularly with strains that have been maintained for a long time in the laboratory away from any contact with tsetse. The strain of *T. gambiense* described here as "Hamburg Braun" I brought from Prof. Reichenow's laboratory in Hamburg to London, together with an old strain of *T. brucei* and of *T. rhodesiense*. For a few weeks in London before my return to Uganda they were looked after by Prof. J. G. Thomson, who kindly arranged for their shipment together with two strains of *T. gambiense* and one of *T. rhodesiense* from his laboratory. These six strains accompanied me on my return to Uganda at the end of 1931, but unfortunately only the three *gambiense* strains reached Entebbe.

Early in 1932 Prof. F. K. Kleine sent by air two guinea-pigs infected with the well-known "Hamburg alt" strain of *T. brucei* from the Koch Institute in Berlin, the strain with which in 1912–14 Prof. Schilling carried out his experiments on the transmission of acquired biological characters (Schilling and Schreck, 1930). These guinea-pigs reached Alexandria where they were promptly placed in quarantine and in due course returned to Berlin. Eventually the strain reached Entebbe by the sea route. In December, 1932, Prof. Yorke sent me by sea, under the care of Dr C. R. Lutze-Wallace of the Uganda Medical Service, the Liverpool *T. rhodesiense* and its arsenic-fast variant with which much of the recent chemotherapeutic work of Yorke and his colleagues has been done.

From nearer home there are included in this investigation two strains of *T. brucei* from Dr J. F. Corson of Tanganyika, originally recovered by Mr Hornby, F.R.C.V.S., in Tanganyika Territory, and known as "Hornby's virulent" and "mild" respectively. There are also two Uganda strains. One of these last is a relic of strain V which when first isolated from man in 1926 was characterised by an unusually high degree of transmissibility by *G. palpalis*; the other, strain "Adero," was isolated in January 1933 from the blood of a native who in the course of a routine examination of labourers was found to be suffering from trypanosomiasis. There was nothing striking about this case; the man had not reported sick and was going about his daily affairs. His strain adds yet one more to the list of well-substantiated examples of non-transmissible strains found in nature in man himself.

A brief account of each strain will now be given, followed by a summary of the experiments performed with each arranged in tabular form (pp. 160–1).

In the table a separate line is devoted to each individual test with each strain. The dates are important and show the period during which each animal was exposed to clean flies, *i.e.* the duration of each test. M = monkey; R = rabbit.

No. 1. T. gambiense, *strain V*

First isolated from man early in 1926 and found to possess an unusual degree of transmissibility by *G. palpalis*. On 26. viii. 26 was introduced by cyclically infected tsetse into sheep 161. On 11. xii. 27 this sheep was superinfected with another strain of *T. gambiense*, again by fly bite. On 9. x. 30 the sheep's blood was inoculated into monkey 765, from which, on 16. x. 31, monkey 871 was inoculated. Monkey 886, on which this test was made, was inoculated from monkey 871 on 8. xii. 31.

No. 2. T. gambiense, *strain "Adero"*

Isolated from a native of the Uganda Protectorate on 29. i. 33. This case was detected during an examination of labourers in connection with the issue of permits to enter certain closed areas. The man did not report sick and showed no striking symptoms. Trypanosomes were found in thick stained blood films. He was presumably infected in the Lake Victoria fly area, possibly in the eastern quarter. There was no definite information about the date of his infection.

Nos. 3 *and* 4. T. rhodesiense, *"Liverpool" strain*
(*from Prof. Warrington Yorke*)

Isolated in 1923 from man. The arsenic-fast variant, No. 4, was prepared by Yorke and his collaborators at Liverpool.

No. 5. T. brucei, *strain "Hamburg alt"*
(*from Prof. F. K. Kleine, Berlin*)

This is the strain used by Schilling and Schreck (1930) in their work on the stability of acquired characters in trypanosomes, experiments performed in 1912–14 and published in 1930. They refer as follows to this "Hamburg alt" strain at the time they used it: "seit vielen Jahren in Ratten und Mäusen weitergeimpft worden war." They found that the exceeding virulence for mice of this old laboratory strain was much reduced by passage through *G. morsitans*, so that in these animals it behaved like a freshly isolated strain of *T. brucei* obtained from the blood of a local wildebeest. They noted also that a calf "unter dem antigenen Einfluss vom Stam 'Hamburg alt' wirksame Antikörper gegen diesen Stamm, nicht aber gegen denselben Stamm der einmal durch die *Glossina* gegangen ist ('Hamburg recens') bildet." They found also that the "Hamburg recens" strain (*i.e.* "Hamburg alt" after cyclical passage through *Glossina*) behaved serologically in the same way as the newly acquired wild *T. brucei* from the wildebeest.

These experiments are in several respects remarkable, and they were criticised by Reichenow (1931), who suggested the possibility of the accidental intrusion of a "wild" strain of *T. brucei*. Schilling has recently repudiated this possibility.

In the light of the experiments with non-transmissible strains which are presented in this paper, a remarkable feature of Schilling's work appears to be

the ease with which the old Hamburg strain was passed through tsetse. He gives no details of this transmission.

Prof. Kleine, writing in 1932, tells me that this "Hamburg alt" strain has been already some 30 years in Germany, and this would make it about 10 years old at the time of Schilling's experiments. That it should after so long a time be still readily transmissible by *Glossina* distinguishes this strain from the other old laboratory strains whose transmissibility by tsetse has hitherto been examined.

Schilling and Schreck's conclusion about the modifying effect of cyclical transmission on acquired virulence has recently been confirmed by Lester (1933). But the changes in serological characters exhibited by "Hamburg recens" appear to me of a more fundamental nature than mere alteration of virulence, and there can be no doubt that confirmation of these experiments is desirable.

In this connection it is interesting to note that Lester, in the work already cited, has shown that acquired resistance to human serum survives cyclical passage through tsetse.

No. 6. T. gambiense, *strain "Braun"*
(*from Prof. E. Reichenow*)

Isolated at Hamburg on 25. ii. 20, from a patient from Fernando Po.

No. 7. T. gambiense, *strain "McA"*
(*from Prof. J. G. Thomson*)

Isolated from a European on 10. xi. 21.

No. 8. T. brucei, *strain "Hornby mild"* (*from Dr J. F. Corson*)

The history accompanying this strain reads as follows:

"*T. brucei* (mild)—from Mr Hornby, Mpwapwa—received 29. vi. 31. Recovered in October 1930 from an ox showing few symptoms of illness, and the strain had been maintained in rats. A goat showed infection but recovered spontaneously and guinea-pigs have been refractory. The disease set up in rats is a chronic one, lasting two or three months, but it is tending towards greater virulence."

No. 9. T. brucei, *strain "Hornby virulent"* (*from Dr J. F. Corson*)

The history sent with this strain is as follows:

"*T. brucei* (virulent)—from Mr Hornby, received 29. vi. 31. Originally recovered in April 1927 from a heifer which had travelled through some of the Kahama fly belts. The heifer showed little ill effect from the infection. Since then the strain has sustained fifty passages through rats, guinea-pigs, rabbits and goats. At one time after a long run through rats it became almost monomorphic, but polymorphism was restored by passage through a goat. In 1927 rats were killed in 30 to 40 days and guinea-pigs in 60 to 80 days. A year or

two later rats were dying within the week but guinea-pigs have generally survived more than a month and there is little difference between the pathogenicity of the strain towards guinea-pigs in those days and these. A series of fourteen goats were killed in three to seven weeks each. Mr Hornby also states that so far as he knows the ox of the mild strain had never been in sleeping sickness country and it was suspected that it was infected through some process of serum manufacture. In Kahama the first case of sleeping sickness was found early in 1928."

No. 10. T. gambiense, *strain "Br" (from Prof. J. G. Thomson)*

Isolated from a European on 14. vi. 30. Of this strain Prof. Thomson writes that it looks to him to be a strain "half-way between *T. gambiense* and *T. rhodesiense*."

General summary

1. The following strains produced no infection in any of the laboratory-bred *Glossina* used in their examination; the total number of flies dissected is given for each strain:

T. gambiense, strain V, Uganda, isolated in 1926	548
T. gambiense, strain Adero, Uganda, isolated in Jan. 1933 ...	1642
T. rhodesiense, Liverpool, isolated in Jan. 1933	750
T. rhodesiense, Liverpool, arsenic-fast variant	1166
T. brucei, "Hamburg alt" from Berlin; isolated over 30 years ago	2452

Some of the flies used in testing each of these strains were kept at 95–97° F. during their infecting feeds.

2. *T. gambiense*, strain "Braun," isolated in February, 1920, gave two "gut only" infections in 1137 flies. *T. gambiense*, strain "McA," isolated in 1921, produced one very light infection, of the intestinal tract only, in 1410 flies employed. This infected fly died on the 27th day after its infecting feed.

T. brucei, strain "Hornby mild," isolated at the end of 1930, gave three infections of the intestinal tract only in 1443 flies used. Some of the flies employed on these three strains were kept during their infecting feeds at 95–97° F., but the infected flies came from boxes kept at room temperature throughout.

It will be seen that all the strains hitherto summarised are, as far as these tests are concerned, non-transmissible by *Glossina*, and the majority are no longer capable of infecting even the intestine of the fly.

3. *T. brucei*, strain "Hornby virulent," isolated in April, 1927, from a heifer, and found by Corson in 1931 to be readily transmissible by *G. morsitans* or *G. pallidipes* (or both), 4½ years after its first isolation (Corson, 1932). A month or so later this strain was found at Entebbe to be still feebly transmissible by *G. palpalis* and somewhat more readily by *G. morsitans*, although much less so than in Corson's experiments.

4. Strain "Br," considered by Prof. J. G. Thomson to be a *T. gambiense* showing some resemblances to *T. rhodesiense*, when examined at Entebbe some

three years after its isolation from a European in West Africa, proved to be still infective to both *G. palpalis* and *G. morsitans* though only very feebly transmissible. 4272 laboratory-bred flies were used in the examination of this strain: 74 of these developed infection of the intestine, and only one a gland infection—a *G. palpalis* dying on the 40th day after its infecting feed. Flagellates were numerous in the glands of this fly.

5. The behaviour of the "Hornby virulent" strain of *T. brucei* and of strain "Br" suggests that completion of the cycle in the fly may be delayed beyond the 25–30 days usually sufficient for East African strains, and it is possible that this delay may be a feature characteristic of strains whose transmissibility by tsetse is undergoing reduction. On the other hand, the solitary infective fly obtained with strain "Br" had a heavy gland infection which had in all probability been present at least for several days before the death of this insect.

6. A strain freshly isolated from a native who was infected on or near the northern shores of Lake Victoria failed to infect any of 1642 *G. palpalis* used in its examination, although a number of these flies were kept at 95–97° F. during their infecting feeds.

7. The results of the investigations described in this paper lend some support to the opinion already formed as the result of numerous experiments with the polymorphic group of trypanosomes, namely that *T. brucei* (and, as far as can be seen, *T. rhodesiense*) is less prone than *T. gambiense* to lose touch with *Glossina*.

It may be that the stability of this character in *T. brucei* is an expression of a more perfect adjustment to environment than is possessed by *T. gambiense*; the latter trypanosome, which is essentially dependent on man, having not yet attained biological equilibrium in this its principal mammalian host.

ACKNOWLEDGMENTS

It is clear from this account how great is my debt to those who have contributed to the collection of the strains for this investigation. In every instance the assistance so willingly rendered involved both trouble and expense, and to all those already mentioned I wish to express my appreciation for their generosity and help. Also to Dr W. H. Kauntze, M.B.E., who, as Deputy Director of Laboratory Services, Nairobi, supervised the strains that came by sea during their journey up through Kenya, and latterly as Director of Medical and Sanitary Services, Uganda, has always been ready to assist the Institute both personally and through the agency of his staff throughout the Protectorate. Lastly, I wish to thank my colleague, Dr J. M. Wallace, who has relieved me of the arduous responsibility of maintaining these strains throughout the period covered by these investigations.

Summary of experiments

Number of strain	Infected animal	Date	Total flies dissected	Total infected	% infected	Flies alive at 25th day: With infected glands	Flies alive at 25th day: Total	Transmissibility index	Total flies used to test strain: Dissected	Total flies used to test strain: Infected	Remarks
1	M 886	15. ii.–6. iii. 32	548	0	0	0	0	0	548	0	
2	M1085	16. ii.–4. iv. 33	641*	0	0	0	0	0			* 275 of these flies were kept at 95–97° F. during the infecting feeds.
	M1107	8–11. iv. 33	84	0	0	0	0	0			
	M1125	28. v.–21. vi. 33	648	0	0	0	0	0			
	M1139	16–23. vi. 33	269	0	0	0	0	0	1642	0	
3	Various	1931 and 1933	750	0	0	0	0	0	750	0	Liverpool *T. rhodesiense.*
4	M1056	17. xii. 32–9. i. 33	606	0	0	0	0	0			Liverpool *T. rhodesiense* arsenic-fast variant.
	M1061	26–29. xii. 32	100	0	0	0	0	0			
	M1064	5–16. i. 33	247	0	0	0	0	0			
	M1079	25. i.–7. ii. 33	213	0	0	0	0	0	1166	0	
5	M1037	14–23. xi. 32	265	0	0	0	0	0			
	R B	24. xi.–10. xii. 32	258	0	0	0	0	0			
	M1047	30. xi.–5. xii. 32	315	0	0	0	0	0			
	M1042	2–19. xii. 32	632	0	0	0	0	0			
	M1054	13. xii. 32–9. i. 33	614	0	0	0	0	0			
	M1066	12–26. i. 33	368	0	0	0	0	0	2452	0	
6	M 866	19. xi. 31–17. ii. 32	625	0	0	0	0	0			
	M 867	3–14. xii. 31	236	2†	0·8	0	1	0			† These two flies died on 18th and 31st day after infecting feed.
	M 868	6–11. i. 32	127	0	0	0	0	0			
	M1076	3–9. iv. 33	149	0	0	0	0	0	1137	2	
7	M 877	14. xii. 31–15. ii. 32	523	0	0	0	0	0			
	M 887	26. xii. 31–15. ii. 32	484	1‡	0·2	0	1	0			‡ A very light infection of the gut in a fly dying on 27th day.
	M 870	16–22. ii. 32	206	0	0	0	0	0			
	M1077	3. ii.–9. iii. 33	197§	0	0	0	0	0	1410	1	§ Flies kept at 95–97° F. during their infecting feeds.

Summary of experiments (continued)

8	M 872	19. xi. 31–17. i. 32	653	0	0	0	0	0			
	M 875	27–29. xi. 31	101	0	0	0	0	0			
	M 882	14–22. xii. 31	135	0	0	0	0	0			
	M 918	16–24. iii. 31	223	1*	0·4	0	1	0			* A 30-day fly (*G. morsitans*).
	M 943	5–14. viii. 32	174	0	0	0	0	0			
	M 943	5–14. viii. 32	157	2†	1·2	0	2	0	1443	3	† Both 38-day flies.
9	M 864	13–22. xi. 31	246	3	1·2	1	1	1·2			
	M 865	16–23. xi. 31	185	1	0·5	0	1	0			
	M 873	24. xi.–11. xii. 31	388	4	1·0	0	0	0			
	M 874	26–29. xi. 31	104	4	3·8	0	2	0			
	M 897	23. i.–1. ii. 32	231	4	1·7	0	3	0			
	M 901	31. vii.–11. viii. 32	245	19	7·7	3	8	2·9			(*G. morsitans*)
	M 901	31. vii.–11. viii. 32	279	5	1·7	0	2	0			(*G. palpalis*)
	M 977	17–23. viii. 32	142	7	4·9	0	2	0			(*G. morsitans*)
	M 977	17–23. viii. 32	68	6	8·8	0	3	0			(*G. palpalis*)
	R 1	7–9. viii. 32	136	11	8·0	1	7	1·1			(*G. morsitans*)
	*	* * * * *	*	*	*	*	*	*	*	*	* *
	M 984	14–22. ix. 32	166	14	8·4	0	5	0			(*G. morsitans*)
	M 984	14–22. ix. 32	193	2	1·0	0	0	0			(*G. palpalis*)
	M 996	20. ix.–13. x. 32	380	7	1·8	0	3	0			(*G. palpalis*)
	M 996	20–25. ix. 32	125	4	3·2	0	0	0			(*G. morsitans*)
	R 27	19–28. x. 32	183	2	1·0	0	1	0			
	R 84	20. x.–2. xi. 32	408	14	3·4	1	3	1·1	3479	107	

Note. The line of asterisks divides the experiments carried out with this strain before (above the line) from those carried out after (below the line) cyclical passage through *Glossina*—the first passage since the isolation of the strain some 5 years earlier.

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A NEW PROTOZOAN PARASITE, *NYCTOTHERUS CHENI* N.SP. (CILIATA), FROM CHINESE FROGS

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(With 2 Figures in the Text)

Introduction

In 1904, Bezzenberger investigated ciliates from the rectum of several amphibian hosts of Asia and described two new species of *Nyctotherus*, *N. macropharyngeus* and *N. magnus*. Recently Dashi Nie (1932) described two more new species, *N. nankingensis* and *N. pyriformis*, with several opalinids from *Rana limnocharis* Gravenhorst of Nanking. It is the purpose of this paper to describe a new species of *Nyctotherus* from the Chinese frog, *Rana spinosa*.

This investigation was carried on at the Zoological Laboratory, University of Pennsylvania, and at the Marine Biological Laboratory, Woods Hole, Massachusetts. To Dr D. H. Wenrich I am indebted for helpful suggestions and criticisms given throughout this work. Also to Mr T. T. Chen for valuable material and for making it possible to secure well-fixed parasites, smears of which were made in China and then sent to this laboratory for staining.

I am indebted to Dr T. H. Cheng of the Fukien Christian University for making some smears in China and sending them to the United States.

The name *Nyctotherus cheni* is proposed for this new species.

Material

Nyctotherus cheni is found in abundant numbers in the rectum of the edible frog, *Rana spinosa*, of Fukien, China. According to Pope (1931) adult and young frogs may be found in great numbers throughout the mountain streams of northern Fukien, from the low coastal ranges of Futsing Hsien to the highest mountains about Kuatun. The embryonic and larval development of *R. spinosa* is said to be extremely slow.

Approximately twenty frogs were examined, nearly all of which were infected with the parasite. In China, the frogs were pithed, opened and placed in Bouin's fluid, and later sent to this laboratory for examination. Besides this new species of *Nyctotherus*, there were also present opalinids and several species of *Balantidium*, all of which were in a good state of preservation.

Method and technique

The parasites were taken from the rectum of the preserved frogs and stained somewhat after the technique devised by Metcalf (1923), while in other instances a centrifuge method was employed for staining. Stains used

were Heidenhain's iron-haematoxylin and Mayer's haem-alum counterstained with eosin. Tuan's method of destaining with a saturated aqueous solution of picric acid was used after the iron-haematoxylin stain (Tuan, 1930). In well-stained preparations, the cytostome appears translucent or colourless, while the nuclear material stains a deep and convincing blue-black colour, making a sharp contrast. This technique seems to be far superior to the usual iron-alum destaining and is used frequently by the writer to show the deeply staining fibrils in other Protozoa. This method was also used for the sectioned material made during this study.

About forty smears on cover-slips were made in China by Dr Cheng from infected *Rana spinosa* and the Protozoa fixed with hot Schaudinn's fluid with 5 per cent. glacial acetic acid. The smeared cover-slips were then placed in a shell vial containing 70 per cent. alcohol and successfully transported to this country, the smears being kept separated by thick paper rings or washers.

Observations and results

The animal seen from the top is generally oval with a thin flattened border or flange approximately 15μ wide, extending out from and encircling the periphery of the ventral surface of the body proper (Fig. 1). For one hundred individuals measured, the average length was 179μ, width 121μ and thickness 63μ. The body proper, dorsally, is irregularly convex, being slightly thicker at the posterior end than at the anterior, where it gradually slopes ventrally until it gracefully meets the flange (Fig. 2). A clear, well-defined ectosarc is found on the body proper. On the ventral surface, the animal is more or less flattened or slightly concave. When seen from above, the protozoon strikingly resembles a man's hat; the flange of the animal resembling the brim of the hat, and the body proper like the crown of the hat (Fig. 1).

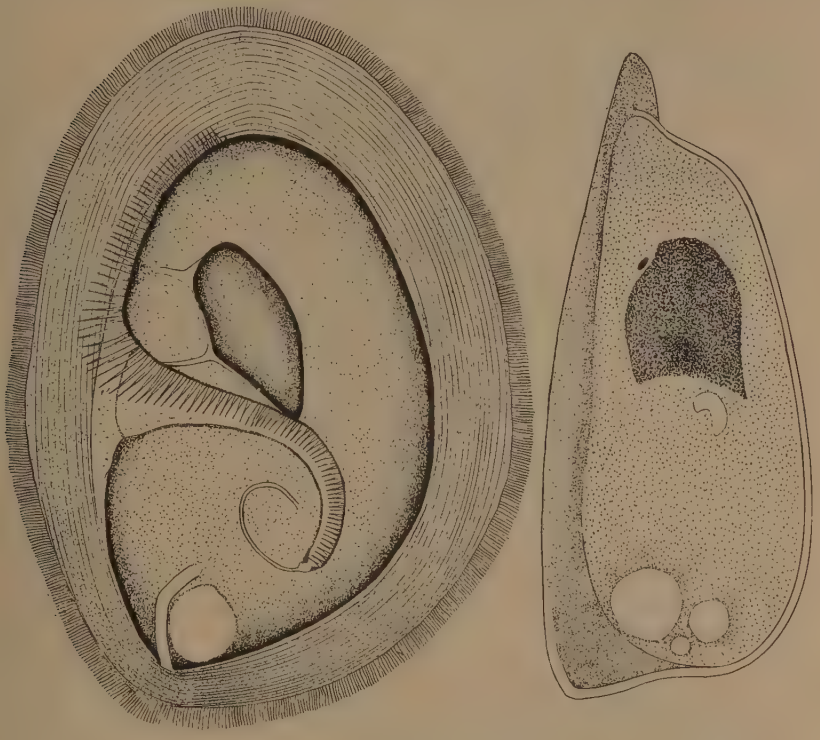
The peristome begins at the centre of the anterior part of the body and extends half-way down its length on the left side where it meets the cytopharynx at right angles. The cytopharynx likewise extends half-way into the body and then takes a broad turn posteriorly. At the end of the cytopharynx may be seen the long coiled subpharyngeal canal (Higgins, 1929), which curves up anteriorly and at times crosses over the cytopharynx and which appears to terminate blindly in the cytoplasm.

Membranelles line both the peristome and cytopharynx, while cilia of moderate length may be clearly observed on the flange which encircles the body proper, the cilia being arranged in definite rows along the ciliary lines. On the body of the parasite are observed ciliary lines with cilia which are not as clearly or prominently seen as on the flange.

At the posterior region of the body is a distinct wide canal or tube which is slightly bent and apparently opens to the outside of the body proper. Near this tube, which is found in nearly every animal, is usually seen a large contractile vacuole; at times there are several smaller vacuoles.

Nuclei

The macronucleus is similar to that of *Nyctotherus magnus* Bezzenberger, in that it is above and close to the cytopharynx and roughly triangular, with the anterior end rounded. When examined dorsally, it measures 49 by 26μ. In well-stained animals (Fig. 1) and in the sectioned material two caryophores or suspensors of the nucleus may be seen (Grassé, 1926). Areas



Nyctotherus cheni. Fig. 1. Dorsal view of the parasite. Fig. 2. Lateral view.

of differentiated protoplasm are observed directly above and below the macronucleus.

The micronucleus, a small oval body, is located in a depression below the anterior part of the macronucleus and takes a much lighter nuclear stain than the macronucleus. The micronucleus is then only visible when the parasite is viewed laterally.

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GLOIOPOTES WATSONI N.SP. AND *LERNAEENICUS SEERI* N.SP., PARASITIC COPEPODS OF FISH FROM CEYLON

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(With 21 Figures in the Text)

(1) **Gloiopotes watsoni** n.sp. (Figs. 1–17)

Host and record. Several males and females were collected from the skin of a sailfish, *Histiophorus gladius* Day, caught off the south coast of Ceylon in December, 1931. Since then this form was found to occur commonly as an external parasite on the skin of many of the sailfish and swordfish found in Ceylon waters.

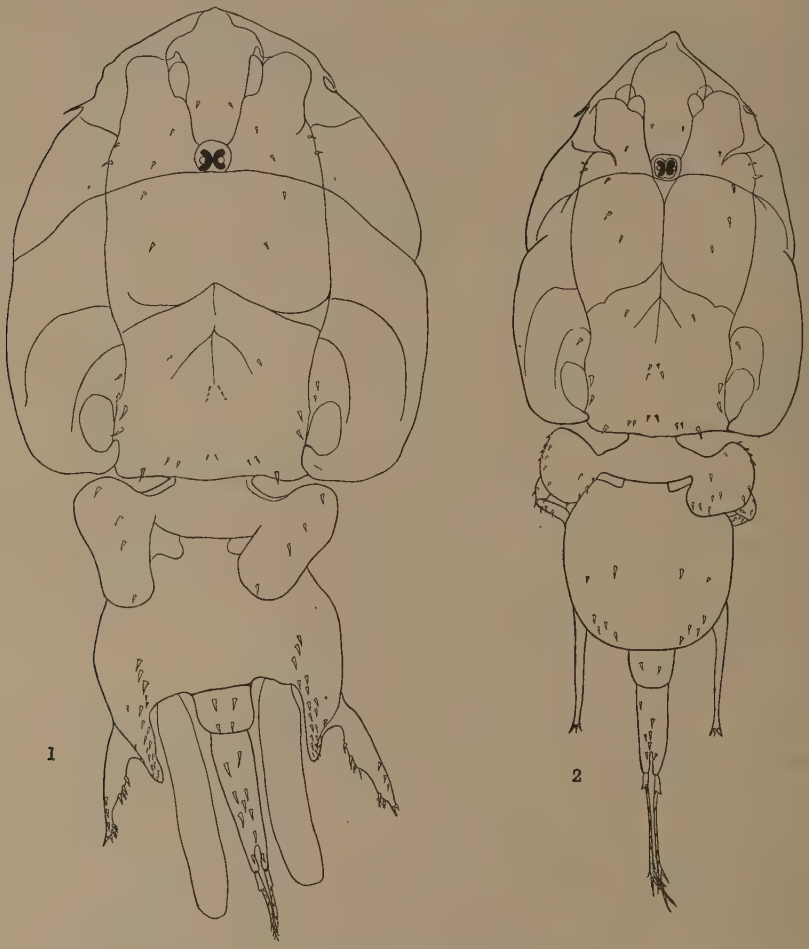
Description of the female (Fig. 1). The carapace, which is about half the entire length, is not much longer than broad and is truncated posteriorly. It is moderately arched. The posterior sinuses are large and deep, the tips of the lateral lobes curving inwards until they slightly overlap the sides of the third segment. The groove separating the head from the thoracic areas of the carapace is well marked, and the eye area is situated in the middle line just anterior to this groove. The eyes are distinct.

The pattern formed by the grooves on the carapace is characteristic. The lateral areas of thoracic segments one to three are marked off by grooves which curve gracefully backwards, and an incomplete longitudinal line divides the second and third median thoracic areas. The transverse groove between these two median areas is also incomplete.

The carapace is furnished with spines which are arranged to form a definite and symmetrical pattern. Beginning from behind the antennae, the lateral margins of the carapace are fringed with a row of hairs; also, three rows of hairs are present on the lateral areas of the head, as well as a row which, starting on the groove between the lateral areas of the head and the thorax, runs backwards on to the lateral area of the thorax along a line parallel with the marginal fringe but a little dorsal to it. This last row of hairs has been figured in *G. ornatus* Wilson, but the three rows on the lateral areas of the head have not been recorded in any of the other species.

The fourth segment is short and triangular and is produced at the sides into a pair of kidney-shaped dorsal plates which are inclined at an angle to the body axis with their concave sides turned outwards. In shape they resemble the dorsal plates of *G. costatus* Wilson but they do not overlap the

genital segment to quite the same extent as in the latter. These plates have a speckled appearance and are furnished with spines, especially along the postero-lateral borders.



Figs. 1, 2. *Gloiopotes watsoni* n.sp. 1, female; 2, male.

The genital segment is more than half as broad as the carapace and is joined to the segment in front by a narrow neck. On either side of the base of this neck is a slight shoulder-like prominence, somewhat in the manner of *G. ornatus*, and from this the sides of the segment curve smoothly backward. Two symmetrical rows of three, sometimes four, stout spines are present on the body of the segment. The posterior lobes extend to about a third of the second abdominal segment and carry a number of small spines on their inner

dorsal areas. The styliform processes project backward and slightly outward. They are somewhat flattened and bear short, stout spines along their margins and at the tips. On the inner edge of each of these processes are present three branched setae, one at about the middle, the other two nearer to the tip of the process.

The two-jointed abdomen has its basal joint short and cylindrical while the much longer terminal joint tapers towards the posterior end where it is markedly bifurcate. The dorsal surface of both joints bears stout spines, in addition to which there are a few small spines on the sides of the second joint which, however, are not so prominent as in *G. ornatus*.

The caudal rami are attached to the bifid extremity of the second abdominal joint and are about half the length of the latter. Their anterior portions for about a third of their length are broader than the remaining two-thirds. Each ramus is armed with spines along its sides and at the apex.

The basal joint of the first antenna (Fig. 3) carries a comb of stout setae; its terminal joint is tipped with three or four smaller setae. The terminal joint of the second antenna (Fig. 4) is a strongly curved hook with a slender spine placed in about its middle towards the antero-ventral edge. The first maxilla (Fig. 5) is a three-parted hook, the two inner prongs fused and smaller than the outer one. The hooks of the second maxilla (Fig. 6) are bifurcate, the outer ramus being longer and larger than the inner. On the outer is a small spine projecting dorsally. The first maxillipede (Fig. 8) is long and slender with the distal joint bifurcate; the second maxillipede (Fig. 9) is a stout hook carrying a small spine at the posterior base of the claw. The posterior rami of the furca (Fig. 7) are bifid while the lateral ones are simple; a secondary furca is present.

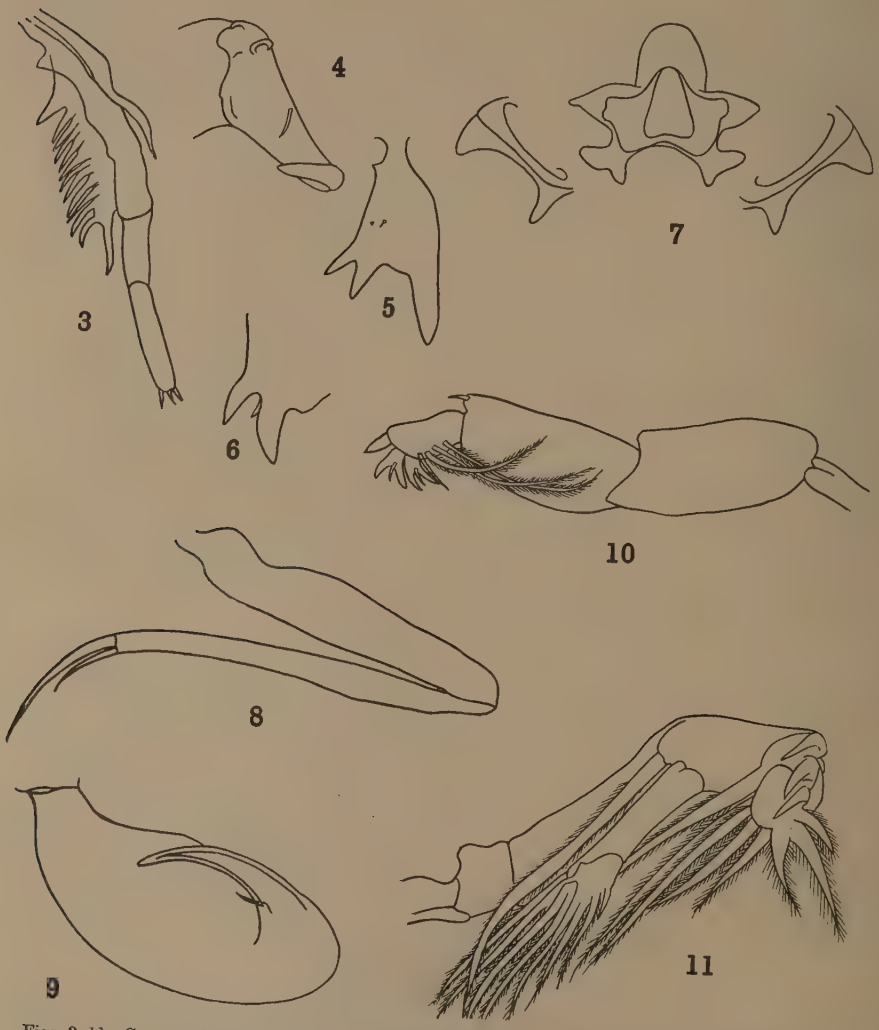
The first leg (Fig. 10) terminates in three claws of which the anterior one is simple; the posterior claws are actually bifurcate although they have the appearance of being three-pronged owing to the presence of a stiff seta at the point of bifurcation of the claws. Wilson has observed that in *G. ornatus* this so-called third prong is slender and remains snowy white even in alcohol while the other two prongs are strongly chitinous. In addition to these three claws the terminal joint carries four processes of which one is simple and the other three pinnate. There is a spine on the distal end of the second joint. The outer ramus of the second leg (Fig. 11) has three simple claws; that of the third leg (Fig. 12) is armed with a large three-pronged claw. The fourth leg (Fig. 13) is long and stout, the three terminal joints are distinctly separated from each other and are together as long as the second joint.

Total length 9·5 mm.; greatest breadth of carapace 4 mm.

Description of the male (Fig. 2). The carapace is distinctly longer than broad and arched rather more than in the female. It is identical with that of the female in the groove pattern, the plan on which the spines are arranged, in the presence of the rows of hairs and in all other respects.

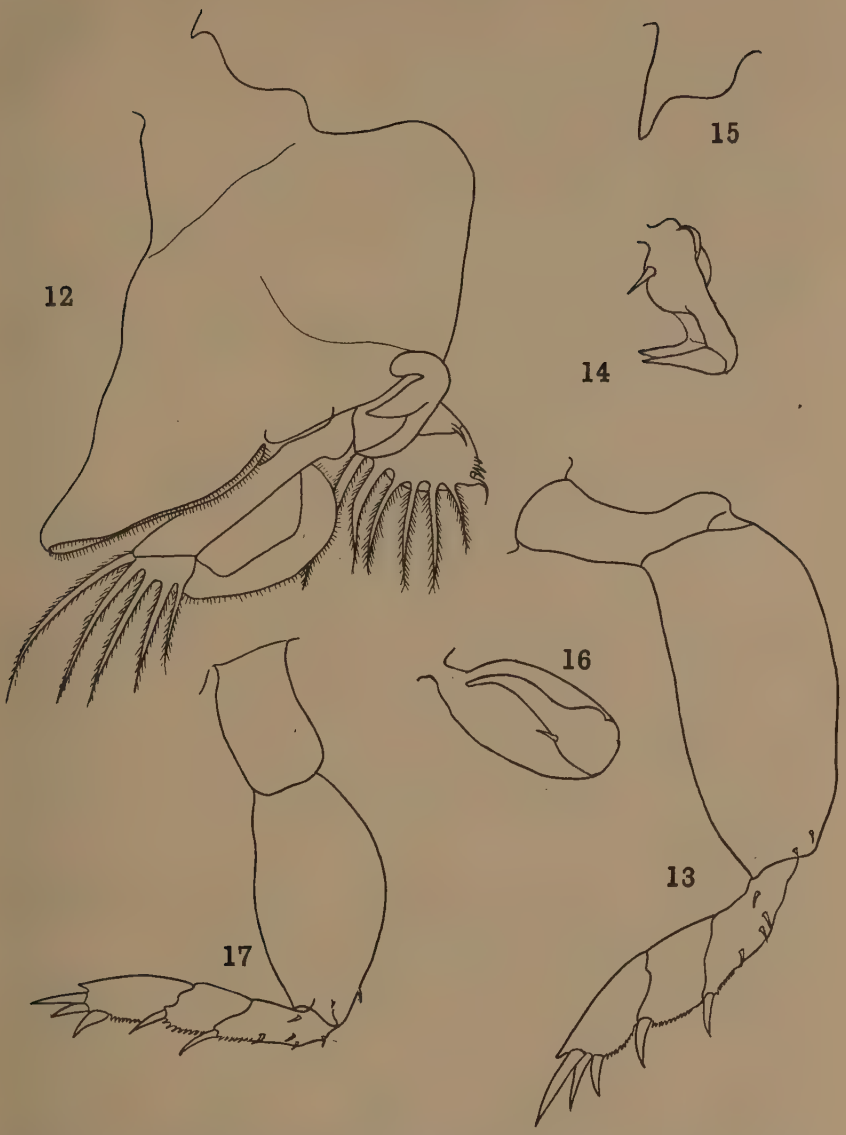
The dorsal plates of the fourth segment, while retaining their speckled

appearance, are not kidney-shaped; neither do they have the rounded form of the dorsal plates of the male of *G. costatus*. Their angle of inclination to the long axis of the body is small, so that they cover very little of the genital segment.



Figs. 3–11. *G. watsoni* ♀. 3, first antenna; 4, second antenna; 5, first maxilla; 6, second maxilla; 7, furca; 8, first maxillipede; 9, second maxillipede; 10, first leg; 11, second leg.

The genital segment is rounded, lacking any suggestion of the shoulder-like prominences of the female, and is provided with spines on its dorsal surface—on each side of the segment two spines, one larger than the other,



Figs. 12–13. *G. watsoni* ♀. 12, third leg; 13, fourth leg.
Figs. 14–17. *G. watsoni* ♂. 14, second antenna; 15, second maxilla; 16, second maxillipede; 17, fourth leg.

near the middle and four spines placed posteriorly. From the genital segment the styliform processes extend backward almost parallel with the abdomen to about half the length of the second abdominal segment and carry three short stout spines at their apices. The second abdominal segment is more than twice as long as the first and is bifurcate at its posterior end to the same extent as in the female. Both abdominal segments have spines on their dorsal surface. The caudal rami, though longer than the second abdominal segment, are not quite as long as the entire abdomen. They are broad at their bases, then become long and slender and are appressed. They are armed with spines dorsally and at the apex.

The second antenna (Fig. 14) carries a spine on the inner margin near its base and the claw is made up of two stout prongs. The second maxilla (Fig. 15) has a single strong spine on its inner edge. The other appendages (Figs. 16, 17) show no marked differences from those of the female except in size and proportions.

Total length 8·5 mm.; greatest breadth of carapace 3 mm.

Remarks. The general body colour of both sexes is a deep violet, with paired red patches showing dorsally. One pair of these patches lies immediately behind the eyes, another lower down the carapace and a third on the genital segment.

This species is well defined by the groove pattern of the carapace as well as the arrangement of the spines on it. Except for the two-pronged claw of the second antenna and the single spine of the second maxilla of the male, the characters of the appendages present no very clear distinguishing features from those of the other species hitherto described. In the shape of the carapace and the dorsal plates the female of *G. watsoni* resembles that of *G. costatus*. In the presence of a row of hairs along the lateral margins of the carapace and in the general shape of the genital segment and abdomen it approaches the condition in the female of *G. ornatus*, but the caudal rami are more like those of *G. huttoni* Thomson.

The collection includes several females without egg-strings. It was at first thought that their egg-strings had been severed from the genital segment when they were picked off the fish, but it was noted later that these forms have a decidedly more flattened carapace than those with the egg-strings, and that in every instance their genital segments had begun to shrivel up.

It is possible that the forms described by Thomson as the males of *Lepeophtheirus* (=*Gloiopotes*) *huttoni* were in fact the old females. The males of all the other species known, *G. hygomianus* Steenstrup and Lütken, *G. ornatus* Wilson, *G. costatus* Wilson and now of *G. watsoni*, definitely confirm Wilson's suggestion that Thomson did not have a male and a female, as he claimed, but only two females, one with and the other without the egg-strings, the latter of which he described as a male.

(The new species here described is named after Professor D. M. S. Watson,

F.R.S., of University College, London, under whose inspiring guidance the author has had the privilege of studying.)

(2) **Lernaeenicus seeri** n.sp. (Figs. 18–21)

Host and record. A single female specimen taken from a seer fish, *Cybium* sp., caught off the south coast of Ceylon in December 1932 is made the type of this new species. The parasite was attached on the right side of the fish close to the anterior edge of the base of the second dorsal fin.

Description. The general form of the body (Fig. 18) is long and slender and the three body regions are distinctly separated. The cephalothorax is more or less cylindrical in shape. At about a third of its length from the anterior end it is constricted by a narrow groove which runs right round it. This groove is so deep at the sides that the connection between the head and the thorax is reduced to a slender median stalk. There is a pair of chitinous horns on each side of the posterior region of the thorax, the two horns of a pair placed one below the other so that all the horns are in the same plane. The right dorsal horn is the smallest, the right ventral is club-shaped due to unequal chitinisation, the left dorsal divides into two short branches at its extremity and the left ventral is the longest.

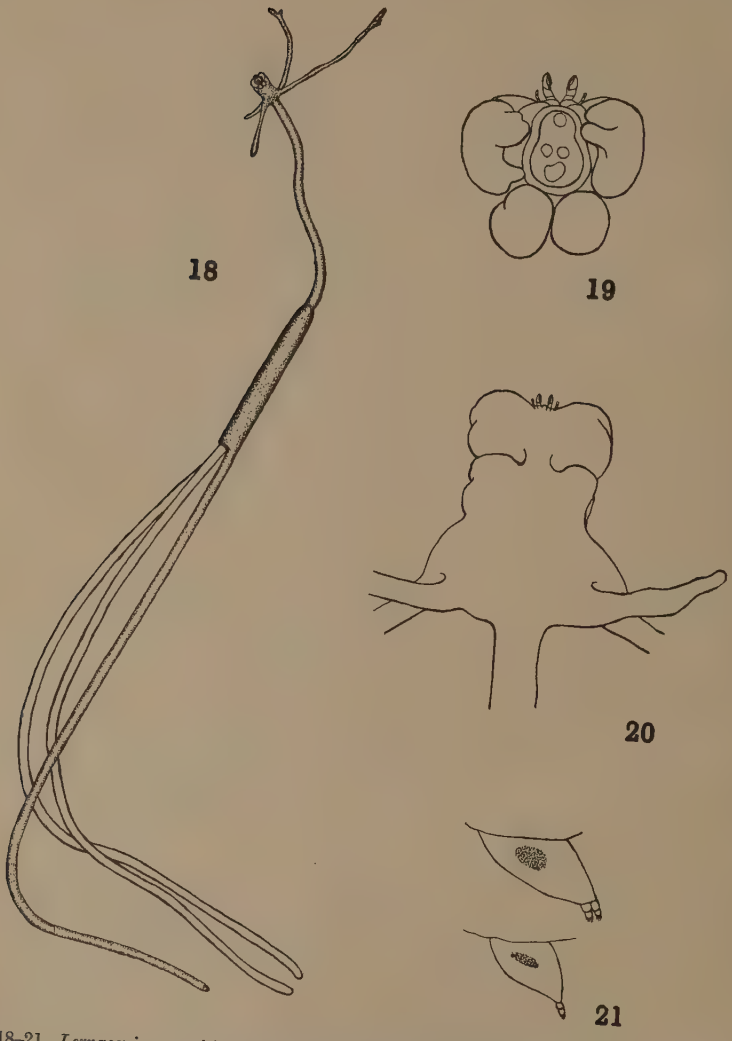
The neck forms very nearly a right angle with the thorax. It is not swollen at its origin but has a uniform diameter until, on approaching the genital segment, it gradually increases in size. A slight constriction marks its union with the genital segment. The latter is not quite as long as the neck, is cylindrical in shape, and has a diameter of twice that of the neck. The abdomen is very long—four and a half times as long as the genital segment. Starting with a diameter of about half that of the genital segment it tapers backwards imperceptibly. The anal laminae are greatly reduced in size and consist of two very small processes on either side of the anus. They are destitute of setae.

The egg-strings (Fig. 18) are a little longer than the long abdomen and contain the disc-like ova in a single series.

The colour of the animal when fresh was a beautiful light blue, with the egg-strings a bluish green, the whole harmonising with the colour of the skin of the host. When preserved in 4 per cent. formalin the body became yellowish white and the egg-strings brown.

The first antennae are short, slender, cylindrical and three-jointed, with scarcely any setae. The second antennae, also three-jointed, are a little longer and stouter. The terminal joint forms an elongate claw shutting down against a small projection on the inner distal end of the second joint.

In the middle of the anterior surface of the head (Fig. 19) is a circular rim within which lies a soft fleshy mass with a few small papillae on it. This mass represents the retracted proboscis. On either side of it is a semicircular swelling, while below it on the ventral side are two round lobes. Due to the



Figs. 18–21. *Lernaeenicus seeri* ♀. 18, entire animal; 19, anterior view of head; 20, dorsal view of head; 21, second and third thoracic legs.

retracted condition of the proboscis the mouth, mandibles and maxillae cannot be made out.

The thoracic swimming legs (Fig. 21) consist of conical basal plates and short, blunt and flattened two-jointed rami. The first two pairs are biramous, the two remaining pairs uniramous. The terminal joint of each ramus is edged with setae.

Dimensions (in mm.): cephalothorax 3; greatest spread of horns 13; neck 17; genital segment 14; abdomen 65; egg-strings 69; entire length (without egg-strings) 99.

Remarks. This species would, in Wilson's key, occupy a position close to that of *L. radiatus* Le Seuer on account of the resemblances they show in (*a*) the radiate arrangement and nature of the horns, and (*b*) the head and thorax being in the same straight line. But here the resemblances come to an end. In *L. radiatus*, although there may be any number of horns from 2 to 9, they are mainly arranged at the anterior end of the head; "there may also be a smaller horn on either side of the base of the second antennae, and two others, one on either side of the thorax on a level with the dorsal surface and opposite the fourth pair of legs." In the single specimen here described as *L. seeri* there are no horns at all anywhere else except on the postero-lateral areas of the thorax. In addition to this, there are many other differences between these two species. They differ in the shape of the cephalothorax. In *L. radiatus* it is globular, while in *L. seeri* it is more or less cylindrical and the deep groove on it is absent on the former. Then again the proportions of the body regions are widely different, the most remarkable feature being the very long abdomen of *L. seeri* which is four and a half times as long as the genital segment. *L. radiatus* has a comparatively stout abdomen which is even less than half the length of the genital segment. In fact no other species has such a long abdomen, the only other form with an abdomen definitely longer than the genital segment being *L. longiventris* Wilson, in which they are in the ratio of 2 : 1. Mainly on account of the length of its abdomen *L. seeri* becomes the longest form yet described within this genus.

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THE ALIMENTARY CANAL OF *CALLIPHORA ERYTHROCEPHALA* L., WITH SPECIAL REFERENCE TO ITS MUSCULATURE AND TO THE PROVENTRICULUS, RECTAL VALVE AND RECTAL PAPILLAE

By G. S. GRAHAM-SMITH, M.D., F.R.S.

(With Plates V–IX, and 24 Text-figures)

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THE writer has recently described in detail the structure and function of the proboscis in *Calliphora erythrocephala* (Graham-Smith, 1930). This paper describes observations on the musculature of the alimentary canal from the pharynx onwards, and the structure and function of the proventriculus, Malpighian ducts, rectal valve and rectal papillae.

I. METHODS

Most of the flies utilised were bred, and material from both newly emerged and older specimens was used. Some of the flies were fed on dilute sugar solution, and killed and dissected at known intervals after meals, others were fed on banana and kept alive for long periods.

For observations on the general structure and relations of the parts and on the epithelial cells lining the canal sections were prepared of whole flies, or of portions dissected out in salt solution, and fixed and stained in various ways.

MUSCULATURE

For the study of the musculature of the alimentary canal specimens were prepared by two methods. The first exhibits the muscles in their normal relations to the other structures composing the wall of the canal, the second exhibits them freed from intima, intestinal epithelium, etc. These specimens were stained by a modification of Heidenhain's iron-haematoxylin method, which gives very satisfactory results.

In order to distend the alimentary canal the fly is allowed to feed on dilute sugar solution until satisfied, and then chloroformed. After momentary immersion in absolute alcohol in order to allow the specimen to sink, it is placed in 0·75 per cent. salt solution, and dissected with straight No. 12 Hagedorn needles fixed in holders.

In the *first method* the required part is transferred on a section-lifter with as little disturbance as possible to a watch-glass containing salt solution, where it is freed from tracheae and other adherent structures, and adjusted in the desired position. In order to lessen the muscular contraction which invariably attends fixation, the greater part of the salt solution is then removed with a fine pipette and the specimen flooded with 5 per cent. cocaine solution for about a minute. It is then fixed in Carnoy's fluid or absolute alcohol for a few minutes, and brought into water. The specimen is then placed in 1·0 per cent. iron-alum solution for a few seconds. Since only the parts mordanted by the iron-alum subsequently retain the stain, and it is desired to stain little else than the outer muscular layers, the

immersion in the iron-alum solution is limited to the period suggested by previous experiments with the structure under investigation. Some shrinkage and hardening occurs during this treatment. After removal from the iron-alum solution the specimen is well washed in distilled water, and then stained in a watch-glass containing 0·1–0·2 per cent. aqueous haematoxylin solution. The results seem to depend largely on the quality and age of the haematoxylin solution. When a satisfactory solution is used staining takes place rapidly, and its progress should be watched under the microscope. If the degree of staining has been judged correctly differentiation is unnecessary. If, however, the specimen has been overstained, it may be differentiated in iron-alum solution to the desired extent, though uneven staining of the muscular strata is likely to result. When suitably stained the specimen is removed to a watch-glass containing water. With the aid of a binocular dissecting microscope the point of a Hagedorn needle, with its cutting edge downwards, is inserted into the lumen of the gut, and the specimen split longitudinally by a succession of short cuts. The specimen is then spread out, the stained muscular surface uppermost, in a drop of water on a slide. At this stage the internal surface of mid-gut preparations, composed of the unstained bodies of the epithelial cells, is opaque and white. The unstained parts become almost invisible after clearing, but the basal portions of these cells, applied to the basement membrane, retain the stain, and appear in mounted preparations as irregular rounded areas beneath the muscle fibres. The flattened preparation, retained under a cover-glass to prevent curling, is dehydrated in alcohol, cleared with clove oil and mounted in balsam. With care considerable lengths of the gut can be split, spread out and mounted in this way.

In successful preparations complicated ramifications of the muscle fibres in the crop, hind-gut and other parts, which cannot be seen in sections, can be followed, and in many instances the differentiated portions within the fibrils can be seen distinctly.

The stained bases of the epithelial cells in the mid-gut and proventriculus and the intima and underlying cells in the fore- and hind-gut interfere, however, to some extent with the observations. For this reason the second method, which removes these hindrances to observation, was devised and extensively employed.

In the *second method* the required parts are dissected out as previously described, and arranged on a section-lifter. They are then immersed momentarily in 25–50 per cent. alcohol, in order to fix the external muscular layer[1], and transferred to a watch-glass of water. The specimens after being split[2] longitudinally along one side are left for periods of time varying from a few minutes to some hours in water and inspected from time to time.

In the case of the mid-gut, if the duration of the immersion in alcohol has been suitable, the epithelium can be brushed off easily, often in large flakes, after a short period of maceration. The brushing is best accomplished with an 00 camel hair brush, from which most of the hairs have been removed. The remaining hairs should be cut down to about 3 mm. in length. A brush with a single hair is convenient for holding specimens or for removing small adherent masses of epithelial cells.

Longer maceration in water is required before the intima can be removed from the hind- or fore-gut. To accomplish this a portion of the intima, some distance from the part chosen for observation, is separated with needles from the overlying muscle and seized with fine forceps. Very frequently the muscular layer can then be separated intact for a

[1] If the parts are overfixed complete removal of the epithelium becomes impossible, while if they are insufficiently fixed they tend to curl up during the operation of splitting and staining is unsatisfactory.

[2] Small tubes, such as Malpighian tubes, heart or aorta, are best split by inserting the tip of a Hagedorn needle with its cutting edge uppermost as far as it will pass without distortion. The back of a second needle is then drawn along the edge of the first, starting from a position near its point, thus severing by compression and without distortion the tissue in contact with the edge. The procedure is repeated as often as may be necessary.

considerable length of the canal by slight traction on the intima and suitable manipulation with a needle. Most of the epithelial cells remain attached to the intima.

Each muscle preparation, when completely isolated from epithelium or intima, is arranged with as little disturbance as possible on a clean cover-glass, partly immersed in the water. Slight pressure with the point of a needle at two or three outlying points causes the specimen to adhere while the cover-glass is very gently withdrawn from the water. The cover-glass, with the specimen uppermost, is then placed on a slide and the specimen examined under the microscope. If necessary, it can be further adjusted, while still covered with a film of water. The greater part of the film of water is then removed with filter-paper, and the preparation kept under observation till the drop in which it lies has almost evaporated. It is then fixed with a drop of absolute alcohol, applied with a platinum loop. Fixation is completed by placing the cover-glass, face downwards, in a watch-glass containing absolute alcohol.

After fixation the specimen is brought to water, immersed in dilute iron-alum solution for 5–20 min., well washed in running water, and then watched under the microscope in a watch-glass containing 0·1 per cent. aqueous haematoxylin solution. Usually a few minutes suffices for staining. The preparation is then washed, passed through the alcohols, treated with clove oil and mounted in balsam. If overstained, differentiation in iron-alum may be necessary.

By this method excellent preparations of the mid-gut musculature can be obtained with ease, but some experience is required in order to obtain satisfactory preparation of the proventricular wall, Malpighian tubes, rectal pouch, junction of the mid- and hind-guts, etc.

Unless otherwise stated the descriptions, which follow, apply to the musculature of the canal, when distended with food, and prepared by the second method.

Thin chitinous membranes, including tracheae and basement membranes, stain a light blue colour and can be studied easily.

The methods used in studying the rectal papillae are stated later.

In fresh preparations the passage of food and movements of the various parts of the alimentary canal were observed.

II. THE REGIONS OF THE ALIMENTARY CANAL

The alimentary canal is divisible into several well-defined regions, oesophagus, crop-duct, crop, proventriculus, thoracic ventriculus, abdominal mid-gut, hind-gut and rectum, and each of these regions exhibits distinct sub-divisions.

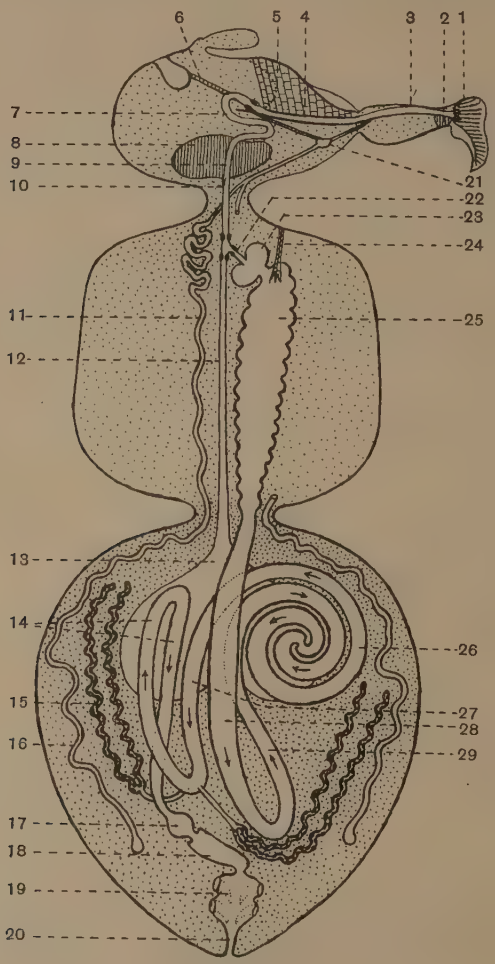
The parts derived from the stomodaeum extend from the pharynx to the centre of the proventriculus, those from the mesenteron form the centre of the proventriculus to just beyond the orifices of the Malpighian tubes, and those from the proctodaeum form the orifices of the Malpighian tubes to the anus.

III. OESOPHAGUS

A. DISPOSITION

The oesophagus can be divided into four portions, which may be called the *pre-ganglionic*, the *ganglionic*, the *post-ganglionic* and the *proventricular* (Text-fig. 1). The crop-duct with its terminal expansion, the crop, is a diverticulum of the oesophagus. The term oesophagus is usually restricted to the

first three portions, since the proventricular portion is most conveniently considered with the proventriculus, and the crop, with its duct, as a separate organ.



Text-fig. 1

(For explanation of Text-figs. 1–24 *see* pp. 238–45)

The *pre-ganglionic* portion extends from the pharynx to the "brain." It commences at the proximal end of the pharynx as a muscular tube, arising dorsally and ventrally from the proximal ends of the dorsal and ventral plates of the pharynx respectively, and laterally from the posterior margins of the inner and outer lamellae. Near its origin the oesophagus is trumpet-shaped, but it soon narrows into a tube of nearly uniform calibre.

The pre-ganglionic portion is free to accommodate itself to altering conditions. When

the proboscis is retracted this region of the oesophagus first curves backwards between the posterior cornua of the pharynx, and then takes a straight course to the "brain" (Graham-Smith, 1930, Fig. 13, III), but when the proboscis is extended a second loop is developed and its general course becomes S-shaped (Text-fig. 1).

The only extrinsic muscles are small bundles of fibres, the retractors of the oesophagus (Graham-Smith, 1930, p. 70), arising from the lower part of the frontal sac and inserted into the wall of the oesophagus a short distance from the pharynx, which serve to draw forward the loop of the oesophagus, when the proboscis is retracted with the head capsule.

The *ganglionic* portion is embedded in the "brain," but is only very slightly attached to its capsule, for it is easy to draw the oesophagus through the brain after severing either its pharyngeal or thoracic end. This portion, according to Patton and Cragg (1913), is narrow in *Philaematomyia*, and Giles (1906, p. 183) states that in *Stomoxys calcitrans* "the muscular and chitinous coats are both so thin as to be barely perceptible."

The *post-ganglionic* portion, commencing at the posterior margin of the "brain," passes through the neck into the anterior region of the thorax, lying throughout dorsal to the great nerve trunk. In the neck an air sac lies on either side of it and the aorta dorsal to it. In the thorax first the aorta and then the proventricular ganglion are attached to it dorsally.

Below the proventriculus the oesophagus divides, one branch, named by Lowne (1895, p. 407) the *proventricular*, passing dorsally into that organ, and the other, which continues the line of the post-ganglionic portion, passing through the thorax into the abdomen as the *crop-duct* (Text-figs. 1, 7).

B. STRUCTURE

Except where the muscular walls are thickened to form sphincters, at the trumpet-shaped commencement and near the bifurcation, all parts of the oesophagus exhibit the same structure.

The inner lining consists of a thick chitinous membrane, the *intima*, which in the resting state is thrown into strong, inwardly projecting, longitudinal folds. When the tube is full of food these folds are to a great extent obliterated. The intima lies on low cubical cells with rounded nuclei, resting on a thin basement membrane. These cells, which are of hypodermal origin, secrete the intima. Externally the tube throughout its length is covered by a muscular coat, which, except in the trumpet-shaped portion and near the bifurcation, consists of a uniform, circularly disposed single layer of muscle fibres. Delicate elongated strands pass from the muscle sheath to the basement membrane. These strands allow the inner coats to be thrown into folds and to slide under the muscle coat in the longitudinal direction to a limited extent.

In specimens removed by dissection, but not split open, the positions of the sphincters can be determined, and the general arrangement of the fibres can be seen in specimens prepared by the first method, but for an accurate knowledge of their disposition recourse to the second method is necessary. The intima can be removed without great difficulty.

The fibres surrounding the trumpet-shaped commencement of the oesophagus are arranged in coarse, thick, circular bundles. This differentiated ring seems to act as a sphincter, preventing, when the fly chooses, the regurgitation of fluid from the crop into the proboscis. It may be termed the *anterior sphincter of the oesophagus*. Stuhlmann (1907) describes a similar sphincter in *Glossina*, consisting of a ring of thick bundles a short distance from the pharynx.

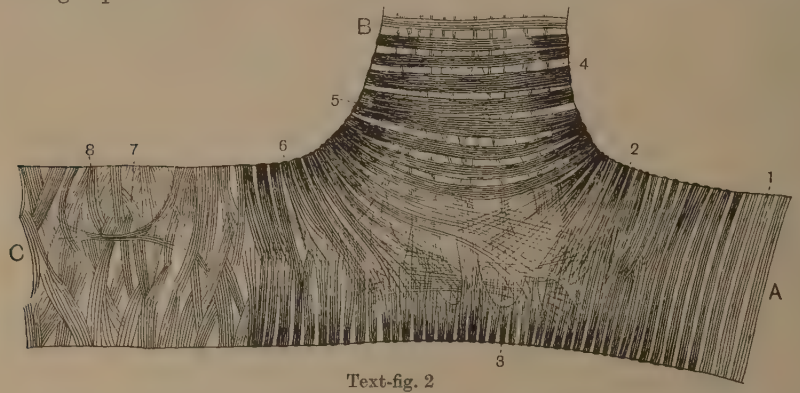
In the terminal portion of the post-ganglionic region the circular fibres become aggregated into coarse bands, which interlace to some extent, forming a *posterior sphincter of the oesophagus*. Beneath the proventricular branch the fibres are irregularly disposed, but occasionally converging bands unite to form bundles of longitudinal fibres, which run for short distances and again divide, and spreading out unite with the circular fibres. There is no definite layer of longitudinal fibres (Text-fig. 2).

Immediately after the bifurcation thick, coarse bundles of circularly disposed fibres are found surrounding both branches, the proventricular and the crop-duct. These rings of coarse fibres act as sphincters, and may be termed the *sphincter of the proventricular oesophagus* and the *sphincter of the crop-duct* respectively. Stuhlmann (1907) briefly describes the proventricular sphincter in *Glossina*.

In a large specimen the oesophagus as far as its bifurcation measures 3·05 mm. in length, the pre-ganglionic portion measuring 1·25 mm., the ganglionic 0·35 mm. and the post-ganglionic 1·45 mm.

C. The functions of the sphincters in the neighbourhood of the proventriculus

Some of the functions of the sphincters in the neighbourhood of the proventriculus can be demonstrated by dissection, others can be deduced from feeding experiments.



Text-fig. 2

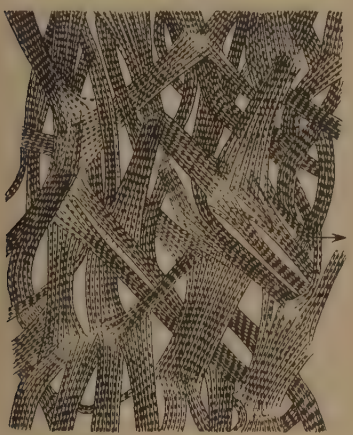
When an anaesthetised fly, previously fed on syrup containing a few particles of lamp-black, is fixed on its side under salt solution in a shallow dish, and the muscles and other structures covering the ventricular region removed, movements of the alimentary canal continue. Frequently masses of pigment may be seen passing forwards and backwards under the proventricular portion of the oesophagus as the crop and crop-duct contract and relax. At intervals the proventricular sphincter relaxes and some of the fluid containing masses of pigment passes with great rapidity into the proventriculus before this sphincter again contracts. While these events are occurring contraction of the anterior sphincter of the oesophagus presumably prevents the escape of fluid through the proboscis, such as occurs when the fly "vomits." Sometimes the posterior sphincter remains contracted and limits the forward movement of fluid to the region immediately under the proventriculus. Since the crop is a reservoir for liquid this sequence of events probably occurs at frequent intervals between meals. Occasionally the crop-duct sphincter remains contracted and then no fluid passes to and fro under the proventriculus, but the pulsations of the crop-duct and the movements of fluid within it cease at the level of this sphincter.

In flies killed after being allowed to feed for a very short time on coloured syrup, the fluid is often found in the crop only and not in the ventriculus. Presumably during this type of feeding the posterior oesophageal and crop-duct sphincters are relaxed and the proventricular sphincter contracted. During "vomiting," when the direction of the flow of fluid is reversed, the sphincters behave in the same manner. On the other hand, it occasionally happens in flies fed on semi-solid faeces that the material passes directly into the thoracic ventriculus and little or none passes into the crop. In this case the posterior oesophageal and the proventricular sphincters must be relaxed and the crop-duct sphincter contracted.

The arrangement of the sphincters is therefore such that, as in a three-way tap, fluid can be made to pass in any direction, except presumably from the ventriculus back into the oesophagus.

IV. THE CROP-DUCT

The crop-duct, continuing the line of the oesophagus, runs backwards below the ventriculus and above the thoracic ganglion in the mid-line of the thorax. Leaving the thorax it passes though the narrow orifice in the first abdominal segment, where it is adherent to the accompanying air sacs, into the abdomen.



Text-fig. 3

Text-fig. 4

Within the thorax the crop-duct is a tube of uniform calibre, capable of considerable distension when full of food. In structure it only differs from the oesophagus in the remarkable disposition of its muscle bands. Near its origin the muscle fibres of the crop-duct are arranged in coarse, rounded, encircling bundles, which constitute the *sphincter of the crop-duct* (Text-fig. 2). Soon the bundles tend to become flatter, wider and more obliquely disposed and to interlace and fuse with neighbouring bundles. Gradually this tendency becomes more marked until in the posterior three-fourths of the thorax the musculature consists of flat, branching, interlacing bands. Preparations of this part consequently present a braid-like appearance (Text-fig. 3 and Pl. V, fig. 1). It is difficult to follow the fibres to

their terminations, but the bands undoubtedly pass under and over each other, and sometimes spread out, the fibres apparently taking origin from the sheaths of neighbouring bands. As in the post-ganglionic portion of the oesophagus converging fibres occasionally unite to form longitudinal bands, which run for short distances. Sooner or later the fibres composing the longitudinal bands diverge to form oblique bands, which unite with circular bands. No distinct longitudinal layer exists.

After passing into the abdomen the crop-duct gradually widens and ends in a rounded, blunt extremity. A short distance from its extremity there arises from each lateral aspect of the tube a large diverticulum with a narrow neck, or peduncle, which constitutes a lobe of the crop.

Within the abdomen a change takes place in the musculature of the crop-duct, the short irregular bands being replaced by longer oblique bands, often with longitudinal branches. On the ventral side many of the branches unite to form longitudinal bands, which pass backwards over the blunt end of the crop-duct, while the oblique lateral bands pass on to the peduncles of the lobes to form their encircling bands. On the dorsal side the arrangement of the muscles is somewhat similar, but the longitudinal bands divide into many branches, which by subdivisions and unions form a complex network. Beyond the network longitudinal bands are re-formed, which are continuous with those which pass round the blunt extremity from the ventral side (Text-fig. 5).

There are relatively very few transversely disposed fibres covering that part of the duct which lies between the peduncles, and these mainly arise as branches of the network.

V. THE CROP

The wide terminal portion of the crop-duct with its two large lateral diverticula, or lobes, constitutes the crop.

When the crop is filled to its utmost capacity with liquid food the lobes appear as smooth, rounded, dorsally directed sacs, which communicate by their necks, the peduncles, with the tensely distended terminal portion of the crop-duct. The mesial walls of the sacs are separated by a deep cleft, continuous with the general abdominal haemocoele (Text-fig. 5).

The lobes are lined, like the crop-duct, by a thick, chitinous intima which lies on flattened cells (Text-fig. 6 E; Pl. V, fig. 4) supported by a basement membrane. The musculature overlying the basement membrane is remarkable. The greater part of each lobe is encircled by flat, narrow, nearly parallel muscle bands. On the median ventral surface these bands converge and by uniting give rise to broader bands which pass on to the median dorsal surface. Along the median dorsal region the broad bands break up into a network of branching and anastomosing fibres (Text-fig. 6 A; Pl. V, figs. 5, 6), which, when they re-unite, give origin to the narrow, parallel bands.

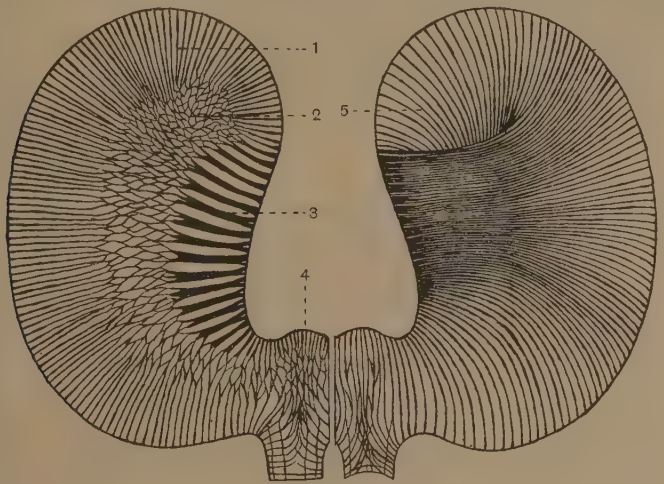
Towards the distal extremity of each lobe the narrow encircling bands gradually assume a longitudinal direction. On the dorsal surface these longitudinally disposed bands unite with the network, but on the ventral surface they meet the circular bands nearly at right angles and are inserted into them (Text-fig. 5).

In satisfactorily stained preparations the arrangement of the muscle fibres can be seen very clearly. Each narrow, flat band (Text-fig. 6 B; Pl. V, figs. 3, 4) seems to consist of a single layer of nearly parallel fibres lying in a sheath with clearly defined edges. At frequent intervals the sheath anastomoses with its neighbours by means of lateral communications, which vary in size from fine strands to channels as broad as, or broader than, the sheath. Most of these communications contain no muscle fibres. In some, however, single muscle fibres or two or more fibres pass from one muscle band to the next, or even to more distant

bands. Usually fibres passing in such communications from one band continue their course in the next, running parallel to the fibres present there. Occasionally, however, a band terminates by its constituent fibres separating and passing either individually, or in groups, by means of the communications to other bands (Text-fig. 6 C).

In the region of the network previously mentioned the large communications are very frequent and irregularly disposed, and in most of them groups of fibres pass in various directions (Text-fig. 6 A, *2*).

The sheath may, therefore, be considered as a closed but perforated sac, which covers the lobe and within which the muscles ramify. Its outer and inner walls fuse along the edges of the bands, except at the communicating channels, thus leaving perforations, which do not communicate with the interior of the sac (Text-fig. 6 B). The outer wall of the sac appears to be the only membrane intervening between the contractile substance and the cavity of the haemocoele. From its inner wall long delicate strands pass to the basement membrane, an arrangement which allows the muscle layer to slide over the inner coats,



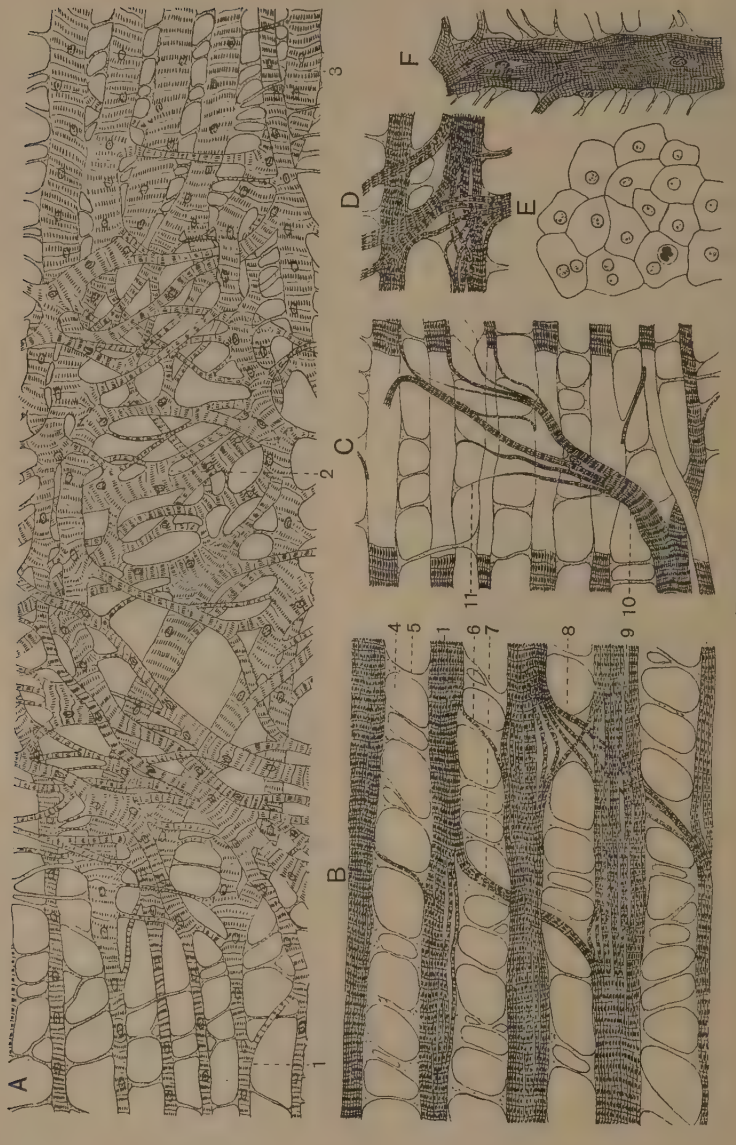
Text-fig. 5

when the crop is expanding or contracting. The disposition of the muscle sheath is similar in most, if not all, other regions of the alimentary canal.

A plexus of nerve fibres ramifies over the muscles together with numerous branches of the tracheae supplying the crop.

Morison (1928), in dealing with the musculature of the honey-bee, describes very similar conditions. He states that "the histology of the splanchnic muscle fibres is characterised by the fibres being more or less flattened against the gut around which they anastomose in an irregular meshwork." He illustrates (Fig. 5, p. 584) anastomosing fibres in the ventriculus, resembling those shown in Text-fig. 6 A, and also parallel fibres in the crop (Fig. 2, p. 580) with apparent communications between their sheaths, which he terms "delicate strands of protoplasmic material passing between the fibres." For the structure of the nerve endings his description should be consulted.

The gradual contraction of the crop may be watched in a fly which has been fed on syrup, chloroformed and dissected under salt solution. Rhythmic contractions of the lobes and duct occur and the lobes gradually diminish in



Text-fig. 6

size and lose their smooth contour[1]. As the contents are forced out irregular constrictions appear in the walls of the lobes, and both lobes become smaller, though not always at the same rate, until their cavities are obliterated. In the same manner the peduncles and finally the terminal portion of the crop-duct become completely contracted.

In the contracted condition the crop often presents an irregular horse-shoe appearance (Text-fig. 4). Dissected specimens show that the intima is thrown into intricate tightly packed folds, but the muscle layer though infolded in many places does not follow the intricacies of the intima.

The function of the crop

At the commencement of a meal the liquid food usually first passes into the crop. After this organ is distended, the food commences to pass into the gut through the proventriculus. Occasionally when flies are feeding on such materials as semi-solid faeces food passes directly into the ventriculus, while little or none enters the crop.

The crop seems to act as a reservoir from which supplies of fluid can be passed into the gut when required.

If hungry flies are given recognisable food and afterwards kept supplied with syrup the remains of the first meal may sometimes be found in the crop several days later. It seems, therefore, that under these conditions the crop is seldom completely emptied.

Blood remains unclotted in the crop. Occasionally large masses of banana, etc., and bubbles of air are drawn into the crop-duct and sometimes enter the crop. Rarely masses of fungoid mycelium have been found growing in the crop, but apparently without affecting the flies.

VI. THE PROVENTRICULUS

A. Relations

Lowne (1895, p. 408) states that the proventriculus "may be described as an intussusception of the stomodaeum into the enteron." He gives a short, but mainly accurate, account of the gross anatomy in *Calliphora*, and mentions that "it is covered by a continuous layer of radiating muscles, which arise from the junction of the oesophagus with the plug." This fact seems to have escaped the notice of other workers, except Stuhlmann (1907), who mentions such fibres in *Glossina* but gives no detailed account of them.

Giles (1906) and Hewitt (1907) briefly described the proventriculus in *Stomoxys calcitrans* and *Musca domestica* respectively, and Patton and Cragg (1913) described it at greater length in *Philaematomyia*. In *Glossina* Giles (1906) gave a short account of it, Stuhlmann (1907) described it in some

[1] When, as often happens, the proventricular sphincter relaxes only at long intervals and consequently the crop contents pass very slowly into the gut, a very small perforation may be made in the crop-duct near the proventriculus so as to facilitate reduction in the size of the crop by the gradual escape of fluid.

detail, and Wigglesworth (1929) directed attention to the probable functions of certain structures within it.

In *Calliphora erythrocephala*, as in other common Muscids, the proventriculus, when viewed from the side after the removal of the salivary glands and other structures surrounding it, appears as a smooth kidney-shaped mass, lying dorsal to the proventricular branch of the oesophagus, which enters it at its ventral pole or hilum. This branch of the oesophagus is directed upwards and slightly backwards, and the proventriculus is tilted correspondingly. Above the proventriculus and in communication with its upper pole lies the thoracic ventriculus. Anteriorly the aorta runs obliquely downwards from the dorsal surface of the ventriculus to pass on to the dorsal surface of the post-ganglionic oesophagus. In the triangle bounded by the oesophagus, aorta and anterior wall of the proventriculus lies the *proventricular ganglion* (Text-fig. 7 C), from which fine nerves pass to the surrounding structures. On each side one larger branch accompanies the proventricular oesophagus into the proventriculus and another accompanies the crop-duct to supply the crop (Text-fig. 7, *e*, *f*). On either side of the proventriculus, above its greatest circumference, lies a small air sac (Text-fig. 7, *m*), from which six to ten tracheae run downwards over the surface of the organ to pass into its hilum. These air sacs are diverticula of the large longitudinal thoracic sacs, lying on each side of the ventriculus.

Viewed from below the proventriculus has a circular outline. In the centre is a deep depression, the *hilum* (Text-figs. 7, *h*; 9, *13*), into which the proventricular branch of the oesophagus passes.

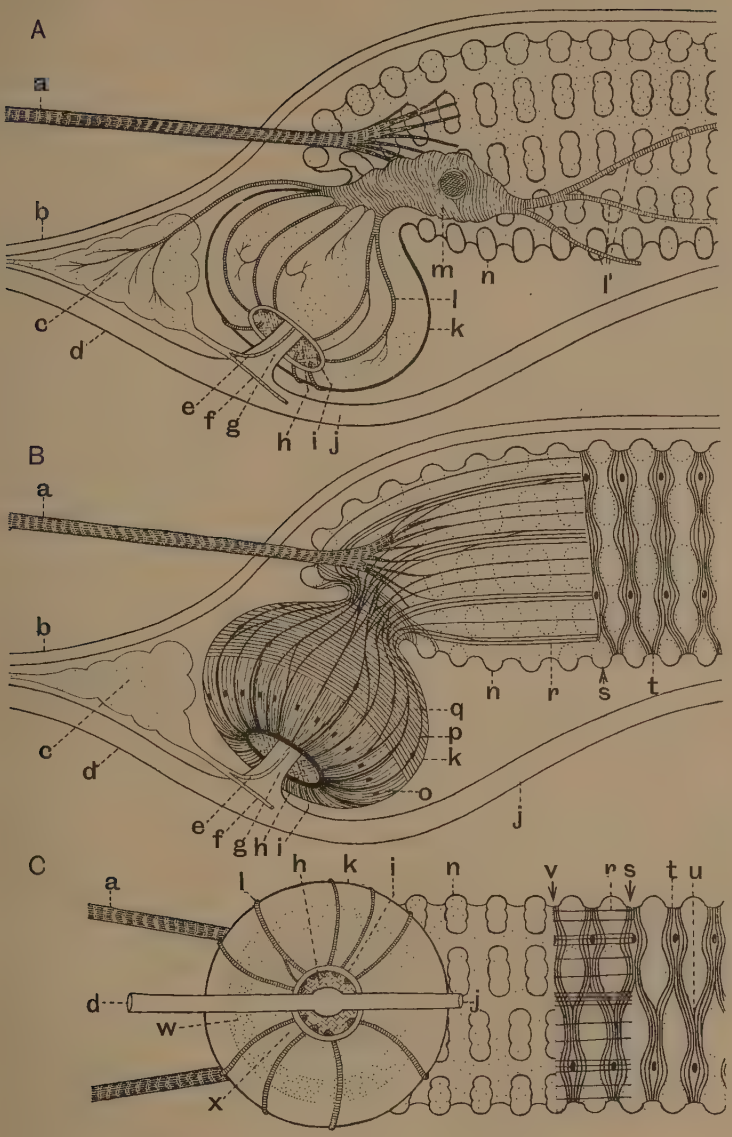
B. Superficial muscles

The whole outer wall of the proventriculus from its junction with the ventriculus to the margin of its ventral hilum represents the proximal end of the mid-intestine expanded over the intussusception of the fore-gut, and is covered with muscles.

The external muscle coat of the proventriculus (Pl. V, fig. 2) commences ventrally as a strong, compact ring of coarse, superimposed fibres encircling the margin of the hilum. Arising as branches from this ring two sets of muscle fibres, deep and superficial, pass upwards. The deep set consists of a thin layer of closely approximated parallel fibres, which pass up to the equator of the organ, where they are lost under circular fibres (Text-fig. 7, *o*). The superficial set consists of parallel bands of fibres, separated by well-marked intervals. Above the equator some of the parallel bands unite to form broader bands, separated by wider intervals. As they approach the upper pole these bands break up into thinner bands, which after various subdivisions and reunions are continued on to the thoracic ventriculus as its longitudinal bands (Text-fig. 7, *p*, *r*).

The vault or upper half of the proventriculus is surrounded by a thin sheet of parallel circularly disposed fibres, which lies deep to the superficial set of longitudinal fibres and seems to end abruptly near the equator, superficial to the extremities of the deep longitudinal fibres. These circular fibres are continued on to the thoracic ventriculus to form its circular bands (Text-fig. 7, *q*, *t*).

Since all these muscles probably assist in the formation of the peritrophic membrane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Text-fig. 7

by pressing the outer wall of the proventriculus against the plug, and possibly assist in driving the food from the proventriculus into the mid-gut, they may be termed the *constrictor muscles of the proventriculus.*

The four different sets of fibres composing the constrictor muscles may be spoken of as (1) the *basal ring muscle,* (2) the *deep longitudinal muscle,* (3) the *superficial longitudinal muscle,* and (4) the *circular muscle of the vault* (Text-fig. 8 A).

No special sphincter is developed at the upper pole of the proventriculus, where it communicates with the mid-gut.

C. Gross anatomy

On dissection the proventriculus is found to consist of two distinct portions, an *outer wall* and an inner "*plug,*" separated from each other by a narrow space, the *cavity* of the proventriculus, except near the hilum, where they unite (Text-fig. 9).

The outer wall, which is continuous dorsally by a narrow neck with the wall of the ventriculus, is thin above the equator, but below it shows on its inner surface two inwardly projecting, well-marked thickenings, separated by a deep groove or cleft. These have been termed the *epithelial pads* (Wigglesworth, 1929). The upper is oval in section, the lower rounded and more prominent. The latter ceases at a point nearly corresponding to the lower margin of the basal ring of the constrictor muscle on the outer surface of the wall. Below the lower epithelial pad the wall again becomes thin and almost immediately unites with the lower flange of the plug.

The whole of the internal surface of the outer wall is lined by epithelium.

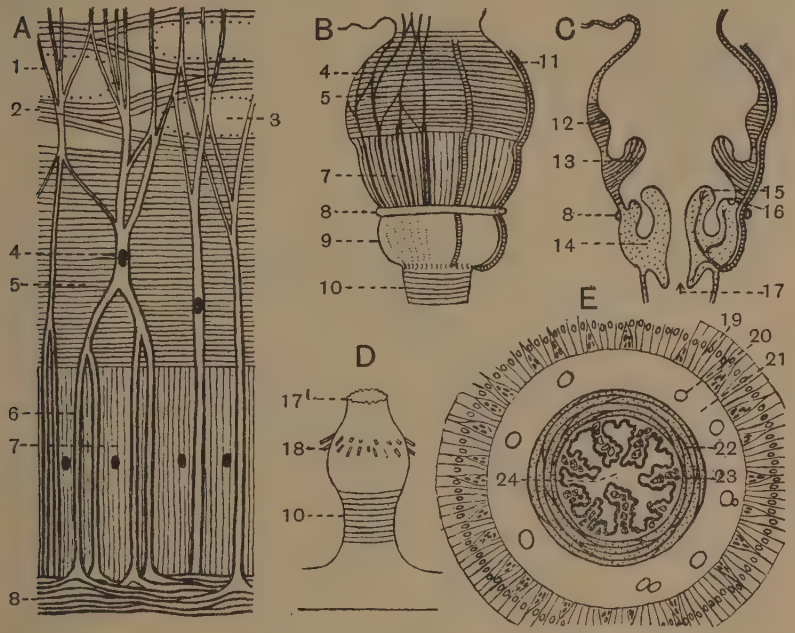
The *plug* (Lowne, 1895, p. 408) is a glistening smooth-walled structure of cartilaginous consistency, covered by a thin sheet of chitin and shaped somewhat like a ship's capstan, with the upper *flange* projecting farther than the lower and with a central perforation passing longitudinally through the stem. It represents the terminal part of the proventricular branch of the oesophagus folded back on itself, so that its chitinous intima has become the external chitinous layer of the plug, and its epithelium has been converted into the cellular tissue of that structure. The upper surface of the plug is round and from the central perforation slopes down on all sides to the overhanging downwardly directed "*rim*" of the upper flange. From the most dependent portion of the rim the wall of the plug returns towards the stem at first upwards, then inwards and downwards, and finally again outwards to form the lower flange. At the ventral margin of the lower flange the chitinous covering ceases and the plug, representing the fore-gut, unites with the outer proventricular wall, representing the mid-gut. The lower epithelial pad of the outer wall fits into the deep groove between the upper and lower flanges.

The proventricular branch of the oesophagus after passing through the hilum proceeds upwards, entering the substance of the plug about the level of the rim of the upper flange.

Some help in understanding the relationship of the parts may be gained by partly reducing the intussusception. This may be accomplished by cutting through the sclerites of the neck and gently drawing the head away from the thorax. The traction of the oesophagus often draws the plug out of the proventriculus (Text-fig. 8 B, C) and allows of clearer observation of structures normally hidden in the hilum. Sometimes this procedure causes the intima of the proventricular oesophagus to tear at the dorsal foramen, where it passes on to the upper surface of the plug. Under these circumstances the proventricular oesophagus only is extracted (Text-fig. 8 D) with fragments of the intrinsic muscles adhering to it.

In fresh whole specimens of the proventriculus examined under the dissecting microscope with suitable illumination the plug can be distinctly seen as an opaque object through the

outer wall and its various parts can be defined. Usually its upper surface seems to be closely applied to the vault. When examined from below an opaque ring is seen surrounding the orifice of the hilum (Text-fig. 7, *x*). This corresponds to the lower flange. Surrounding this opaque ring is a pale zone, itself surrounded by an outer opaque ring (Text-fig. 7, *w*). The pale zone corresponds to the groove in the plug and the outer opaque ring to the rim of the upper flange.



Text-fig. 8

D. HISTOLOGY

The finer details of the structure of the proventriculus are best made out by means of serial, vertical, longitudinal sections stained by various methods, supplemented by sections in other planes.

The proventricular oesophagus takes a straight course to the centre of the plug, and its channel continues straight through the plug to open as the *dorsal foramen* (Text-fig. 9, *32*) immediately below the orifice in the vault of the outer wall leading to the ventriculus. Before entering the plug the proventricular oesophagus is lined by a thick chitinous intima thrown into strong, inwardly projecting, longitudinal folds. The epithelial cells below the intima are well defined. The outer coat consists of bundles of circular muscle fibres, constituting the *sphincter of the proventricular oesophagus*. Some of these fibres unite to form longitudinal fibres, which usually lie in the bases of the folds of the intima (Text-figs. 2 and 8 E).

Within the plug, especially in its upper third, the intima becomes sacculated, though the longitudinal folds still continue. Near the dorsal foramen, however, the intima becomes thinner and closely bound to the subjacent cells, and in this condition is reflected over the whole outer surface of the plug. The sphincter muscle, though diminishing rapidly in amount,

A
B
a
b
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Text-fig. 9

continues to surround the channel to within a short distance of the dorsal foramen, and a few of its longitudinal fibres are continued up to the same region. Other muscle fibres radiate outwards into the substance of the upper flange of the plug. These may be termed the *intrinsic radial muscles of the plug* (Text-fig. 9, *33*). Yet other fibres originating near the central region of the upper flange pass longitudinally downwards in the substance of the plug as far as the point of junction of the lower flange with the outer wall. Their prolongation beyond this point is difficult to demonstrate, and has been omitted in Text-fig. 9, but in some preparations fine wavy fibrillae seem to reach the ring fibres of the constrictor muscle (see Text-fig. 8 B, *9* and compare with Text-fig. 13, *f*). They may be termed the *intrinsic longitudinal muscles of the plug.*

Beneath the external chitinous layer of the plug lie closely packed, pale staining, columnar cells with oval nuclei, ending deeply in long branches, which pass into the substance of the plug to form a network. In the middle line of the outer part of the upper flange the branches of the cells of its two surfaces seem to meet around the intrinsic radial fibres, but elsewhere the branches pass ventrally and apparently fuse to form a membrane, which constitutes the dorsal and outer walls of the hilum. It is possible, however, that the interspaces of the network represent deep grooves to accommodate the muscles and tracheae, and that the apparent membrane is due to secondary unions of the external borders of the grooves. Whatever the explanation, the fibres of the intrinsic muscles, both radial and longitudinal, in company with numerous terminal branches of the tracheae, run in the interspaces of the network (Text-fig. 9 A, B, C). As they approach the periphery of the plug the intrinsic fibres divide into fine branches which appear to be inserted into the bases of the cells, but may reach the chitin.

The junction of the lower flange of the plug with the external wall is indicated by the chitinous lining of the plug passing deeply to meet the basement membrane of the wall, and to separate the characteristic epithelia of the two regions.

The outer wall of the proventriculus is composed of a single layer of epithelial cells lying on a basement membrane, covered externally by the constrictor muscles already described. In the dorsal or vault region the epithelium, which is continuous with that of the thoracic ventriculus, consists of cubical deeply staining cells. Towards the equator the cells gradually become columnar and then again diminish in height. This region of columnar cells forms the upper epithelial pad, and the subsequent region of cubical cells the cleft between the two pads. Below the cleft the cells again become elongated to form the lower epithelial pad. In the centre of the lower pad the cells are very tall and attached to the basement membrane by thin stalks. Beyond the centre of the pad the cells again diminish in height, but columnar epithelium is continued up to the junction of the wall with the lower flange of the plug.

The cells forming both epithelial pads stain deeply and contain vacuoles and globules. Also throughout these regions the free extremities of the cells exhibit a clear or striated zone. In the cubical cells of the vault the vacuoles are few and the clear zone small or absent.

The *peritrophic membrane* lies between the outer wall of the proventriculus and the plug, wholly enclosing the surface of the latter structure. In sections it may be traced downwards from the ventricular opening as a thin lamina between the outer wall of the vault and the upper flange of the plug. Nearly opposite the rim of the upper flange it divides (Text-fig. 9, *26*), one division passing to the neighbourhood of the base of the cleft between the epithelial pads, the other following the surface of the lower epithelial pad to the neighbourhood of the junction of the outer wall with the lower flange of the plug.

The *hilum* is a deep cavity in the base of the proventriculus, surrounding the proventricular branch of the oesophagus and communicating freely with the thoracic haemocoele. Its lower limit is defined by the inner margin of the ring fibres of the constrictor muscle. Its roof and outer boundaries are formed by the "membrane" covering the intrinsic longitudinal muscles and its inner boundary by the outer coat of the proventricular oesophagus.

Besides the tracheae which traverse the hilum, a few fat cells are usually present in its upper part. In some Muscids, such as *Glossina* and *Stomoxys*, these cells are sufficiently numerous to fill, or nearly fill, the cavity, making its recognition difficult.

The *tracheae*, which have been noted previously (p. 188) running downwards from the air sacs superficially to the constrictor muscles (Text-fig. 7 A), may be traced farther in such sections. These tracheae pass deep to the basal ring fibres of the constrictor before entering the hilum (Text-fig. 9, *27*), and run upwards in the cavity of the hilum (Text-fig. 8 E) to plunge into the substance of the plug near its fusion with the oesophagus (Text-fig. 9, *34*). After entering the substance of the plug the tracheae divide. The largest branches accompany the intrinsic radial muscle fibres outwards towards the rim of the upper flange. These branches break up into very numerous smaller branches, which form a fine arborescence, especially in the neighbourhood of the rim. The terminal branches of the arborescence do not seem to anastomose. By dissecting out the plug in a fresh specimen, cutting through its stem and mounting the whole upper portion in saline under a cover-glass with slight compression, the distribution of the tracheae to the upper flange can be beautifully seen. Other smaller branches accompany the proventricular oesophagus upwards and others pass downwards in company with the intrinsic longitudinal muscle fibres to the lower flange.

The proventriculus of *Musca domestica* very closely resembles that of *Calliphora erythrocephala*. In *Stomoxys calcitrans* the upper flange is flatter and the lower flange and the upper epithelial pad relatively less developed. Large fat cells in the hilum obscure its cavity. By the kindness of Mr L. Eastham the writer was afforded the opportunity of studying a preserved specimen of *Glossina morsitans* and also longitudinal sections of the proventriculus. This material was sufficient to show that in *Glossina* most of the structures found in the proventriculus of *Calliphora* are present. In certain respects, however, the proventriculus of *Glossina* differs from that of the other species studied. It is elongated longitudinally and the sides are folded downwards so that they overlap the bifurcation of the oesophagus. "As seen from below, it has much the form of a Spanish priest's hat" (Giles, 1906, p. 185). Consequently longitudinal and transverse sections, unlike those of *Calliphora*, show different appearances. The proventricular branch of the oesophagus is relatively short, and the hilum is elliptical in shape. Anteriorly and posteriorly the cavity of the hilum is of considerable extent, but obscured by the fat cells it contains. Laterally the outer wall of the hilum is closely applied to the proventricular oesophagus. Externally the circular vault fibres as well as the deep vertical and ring fibres of the constrictor muscle are present, the latter being well developed. The superficial vertical set is probably also present, but in preserved specimens the adherence of an air sac to the side of the proventriculus renders it difficult to obtain suitable preparations. Internally the upper epithelial pad is not distinct, but the lower pad is well developed. The peritrophic membrane seems to have a single source of origin. The plug is relatively compressed in the vertical direction, but extended in the horizontal direction, and its upper and lower flanges and the groove embracing the lower epithelial pad are very distinct. The sphincter of the proventricular oesophagus is strongly developed and is continued in the substance of the plug nearly to the dorsal foramen. Intrinsic radial muscles are present in the plug[1].

E. The functions of the proventriculus

(*a*) *The formation of the peritrophic membrane*

Lowne (1895) apparently did not notice the peritrophic membrane in the proventriculus of *Calliphora*, but thought that the cells of the mid-gut "shed from time to time" mucoid material, which separated "the more solid particles

[1] Stuhlmann (1907, Pl. VIII, fig. 56) illustrates intrinsic radial fibres in the plug and also fibres below the hilum, presumably ring fibres of the constrictor seen in longitudinal section, and regards both sets as dilators of the oesophagus as it passes through the plug.

of food from the epithelium." Vignon (1901) showed that in the larva of *Chironomus* the membrane is derived from a secretion in the proventriculus, which becomes hardened after being moulded by pressure between the folds of that organ. Stuhlmann in 1905 thought that it was formed in the proventriculus of *Glossina*, but later (1907) concluded that it was shed off from the surface of the mid-gut. Berlese (1909) in *Calliphora* and Patton and Cragg (1913) in *Philaematomyia* pointed out that, originating in the neighbourhood of the epithelial pads, it appeared to grow into the mid-gut from the proventriculus.

Wigglesworth (1929) investigated its formation by means of "transverse serial sections of the entire thorax of *Glossina* (with the proventriculus fixed *in situ*) at all stages of digestion." He points out that the cells of the epithelial pad contain many clear vacuoles and that numerous globules of secretion are "discharged through the border of the cells." He also points out that the epithelial pad is pressed closely against the wall of the groove in the plug and that the outer wall of the proventriculus is similarly in close contact with the rim of the upper flange. "In fact the copious secretion of the epithelial pad is obliged to pass through the narrow cleft between the proventricular wall and the invaginated fore-gut and in so doing is pressed and moulded to form the peritrophic membrane. The tension in the mid-gut transmitted to the walls of the proventriculus, assisted perhaps by the pressure of the contents of the proventriculus upon the funnel-shaped invagination, will serve to keep the two structures—the rollers of the press—in intimate contact." He thinks that the columnar cells of the walls and vault and perhaps the cells lining the commencement of the mid-gut contribute to the secretion of the peritrophic membrane. He is of opinion that in *Glossina* "it is produced very rapidly during the process of feeding and is drawn through the press by the tension of the advancing blood as silk is drawn through the spinneret of a caterpillar or a spider. The peritrophic membrane is evidently formed afresh in whole or in part each meal." Wigglesworth (p. 312) has shown by various tests that, as is generally assumed, the peritrophic membrane of *Glossina* "is composed of chitin, and, as was to be expected from its recent formation, of chitin which is very readily converted into chitosan."

In *Calliphora erythrocephala* on the other hand the peritrophic membrane extends from the proventriculus to the rectal pouch or even to the anus. Throughout its whole length it forms a strong tube or sheath, without apertures, which separates the food contained within it from the epithelium lining the alimentary canal (Pl. IX, fig. II). It is present fully formed in the newly emerged fly, and, at present, there is no evidence that any further growth, which may occur, is related to the taking of food. It is probable that the membrane is continually being secreted, for its capacity for staining with haematoxylin suggests that it is composed of newly formed chitin.

In suitable longitudinal sections of the proventriculus, as stated previously (p. 193), the membrane appears to arise as two laminae, which unite opposite

the rim of the upper flange. These laminae, when traced downwards over their respective epithelial pads, pass on to and seem to fuse with the free surfaces of the striated borders of the secreting cells. The secretion from each pad seems therefore to be solidified and moulded into a lamina almost immediately, whilst the two laminae are united by pressure near the rim of the upper flange. Free secretion filling the space between the pads and the plug, as described by Wigglesworth in *Glossina*, has not been observed, but may be present in certain stages of digestion.

The fibres of the constrictor muscle seem to be admirably disposed for producing the pressure required for moulding. The fibres of the basal ring and the circular fibres of the vault afford lower and upper fixed regions, and the deep longitudinal fibres acting between them, aided by the superficial longitudinal fibres, compress the roller mechanism. It seems probable that the radial and other intrinsic fibres of the plug render that structure more rigid during the periods of membrane formation. By peristaltic movements the circular fibres of the vault may assist the newly formed membrane to pass into the mid-gut.

(*b*) *Other functions*

Most of the views put forward in regard to the other possible functions of the proventriculus have been founded on the study of its structure and not on observation on living specimens.

Lowne (1895, p. 409), regarding the proventriculus as "homologous with the gizzard of manducatory insects," thought that it might "serve for crushing soft, semi-solid food." He was consequently inclined to think that it was "a gizzard and nothing more." There is no evidence that it has such a function and food seems to pass through it with great rapidity.

Giles (1906), after describing the structure of the proventriculus in *Stomoxys calcitrans*, says: "Taking the structure as a whole, it is difficult to resist the idea that it must, in some way, have a valvular function, though it is difficult to say how." Hewitt (1907, 1914) considered that in *Musca domestica* "its structure suggests a pumping function and also that of a valve." Patton and Cragg (1913, p. 115) remark that in *Philaematomyia* "the structural arrangements at the communication of the oesophagus and the mid-gut are evidently adapted to function as a valve to prevent the regurgitation of the contents of the gut. Pressure of fluid in the anterior end of the mid-gut would result in dilatation of the annular recess" (namely, the recess between the upper and lower flanges of the plug) "and would, therefore, compress the sides of the opening of the oesophagus." Patton and Evans (1929, p. 159), in describing the structure in *Musca domestica*, put forward the same view, stating that "the pressure of fluid food, in the cul-de-sac at the side of the mid-intestine, would close the plug of fibrous cells, which then act as a valve and prevent regurgitation of food from the mid-intestine forward, when the pharynx is in action." Wigglesworth (1929, p. 305) considered that this is the only one of

these hypotheses which "will really account for the complex but constant form of the organ."

In transverse sections through the proventriculus of recently fed *Glossina*, Wigglesworth (1929, p. 305) described a circular muscle in the plug just ventral to the dorsal foramen (which longitudinal sections suggest is the distal portion of the circular musculature of the proventricular oesophagus) and stated that it was clear "that the proventriculus acts as a sphincter and not as a valve."

Wigglesworth later (1930) investigated the formation of the peritrophic membrane in several orders of insects by examining the "oesophageal valve" in many larvae and some adults, and concluded that it "never functions as a valve at all, but always as a sphincter between the fore-gut and the mid-gut, and frequently as a press for the peritrophic membrane....The mechanical efficiency of this press varies greatly from one order to another; and the mechanism attains its most specialised forms in the Diptera" (p. 612).

Observations on living specimens of *Calliphora erythrocephala* described previously (p. 182) indicate that the sphincter of the proventricular oesophagus remains in a state of tonic contraction, preventing regurgitation of food, except at such times as it relaxes to allow food to pass from the oesophagus into the proventriculus. Also in observations on fresh preparations by transmitted light no separation of the vault from the plug, suggesting the presence of food in the organ, has been noticed. There is, therefore, no need to postulate a valvular function, and Wigglesworth's figure (Text-fig. 11, p. 306, 1929) shows that in *Glossina* after a large meal food does not penetrate into the annular recess in the manner suggested by Patton and Cragg.

The evidence at present available suggests that a sphincter in connection with the proventricular oesophagus controls the inward passage of food and prevents regurgitation, and that the function of the rest of the proventriculus is to produce and mould the peritrophic membrane.

VII. THE MID-GUT

A. Disposition

As stated in a previous section (p. 190) the mid-intestine commences at the junction of the outer wall of the proventriculus with the plug, but for descriptive purposes it may be taken as commencing where the vault of the proventriculus narrows and becomes continuous with the ventricular wall.

From this point the mid-intestine runs straight backwards in the mid-line of the thorax, ventral to the aorta and dorsal to the crop-duct, to pass into the abdomen. This intra-thoracic portion may be termed the *thoracic ventriculus* (Text-fig. 1, *25*)[1].

[1] There is so much confusion in the nomenclature of the regions of the mid-intestine that it has seemed desirable to introduce new terms.

Lowne (1895, p. 409) gives the name *chyle stomach* to the portion of the mid-gut of *Calliphora erythrocephala* which extends from the proventriculus to the commencement of the helicoid region. "The thoracic portion is covered by small hemispherical projections, except at its anterior

After entering the abdomen (Text-fig. 10 A) the mid-intestine, following the configuration of the tergites, curves dorsally, though continuing in the mid-line, till it reaches the level of the posterior margin of the apparent third tergite. This portion, which lies ventral to the heart, may be termed the *abdominal ventriculus* (Text-fig. 1, *28*). At the level mentioned the mid-intestine bends sharply and passes downwards and forwards till it almost reaches the ventral wall of the abdomen, where it again bends sharply to pass upwards and forwards below the abdominal ventriculus. This portion, which is nearly equal in length to the abdominal ventriculus, consists, therefore, of a descending proximal limb, a sharp bend, and a dorsally directed distal limb, and may be called the *ventricular loop* (Text-figs. 1, *29*; 10 A, *2*). At the termination of the distal limb of the ventricular loop the intestine curves to the right to form a helix, consisting of one and a half proximal turns to the right, followed by a very acute bend leading to one and a half turns to the left. These coils are very closely apposed and are held in position by numerous tracheae. This portion, which lies ventral to, and mainly to the right of, the abdominal ventriculus, may be termed the *helicoid region* (Text-fig. 1, *26*). The terminal portion of the mid-intestine passing from the distal coil of the helix crosses to the left side under the anterior part of the abdominal ventri-

extremity, which is smooth like the abdominal portion. Hence the chyle stomach may be said to consist of three parts or segments: the caput, the thoracic or intermediate part, and the pyloric extremity or abdominal part." He gives the name *proximal intestine* to the helicoid and post-helicoid regions.

Hewitt (1907, 1914), in describing the anatomy of *Musca domestica*, calls the intra-thoracic portion the *ventriculus* or *chyle stomach*, and the whole of the intra-abdominal portion the *proximal intestine*.

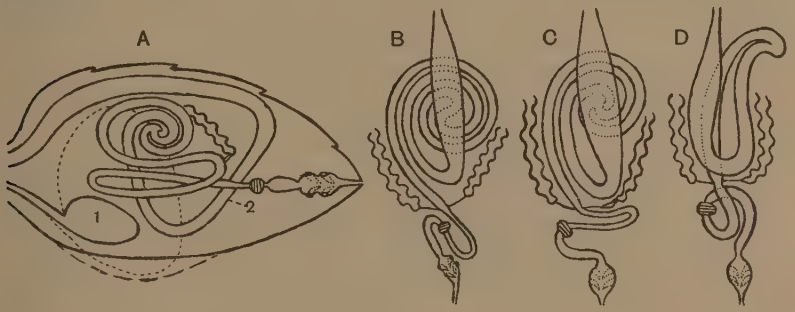
Patton and Cragg (1913, p. 115), describing the alimentary canal in *Philaematomyia*, state that "the mid-gut may be conveniently divided into three portions. The first of these is a narrow tube immediately posterior to the proventriculus, which passes directly through the thorax without convolutions. This expands after a short course to form the portion known as the *ventriculus* or *chylific ventricle*, which is the widest part of the gut; it lies in the posterior part of the thorax and in the abdomen, and is twisted into a double coil in its posterior part. The third part, corresponding to rather less than half the total length of the mid-gut, is known as the *proximal intestine*, a rather unfortunate term; it should not be confused with the hind-gut. It is about half the diameter of the ventriculus, and is more loosely coiled. At its posterior end the Malpighian tubes enter the gut." They say (p. 117) that "the alimentary canal of *Musca* is very similar to that of *Philaematomyia*. The mid-gut is a little longer and more convoluted, and the ventriculus broader. The latter is narrower at its anterior end than elsewhere, but it is not separated from the proventriculus by a definite neck as is the case in *Philaematomyia*, so that almost the whole of the part lies in the thorax."

Patton and Evans (1929, p. 157) state that in *Musca* "the first part is a narrow tube into which the ventriculus leads, and passes straight through the thorax without convolutions. It expands to form the middle portion, generally known as the *ventriculus* or *chyle stomach*; this is the widest part of the mid-intestine, and lies in the posterior part of the thorax, and in the abdomen, in which it is convoluted, forming a double coil at its distal end....The third part is wrongly named the *proximal intestine*, a term which is apt to suggest a part of the fore intestine; this part is narrower than the middle portion and is not so firmly coiled. It joins the hind intestine at the point where the Malpighian tubes enter the alimentary tract."

Wigglesworth (1929) divides the mid-intestine of *Glossina*, in which there is no definite helix, into three histological regions, anterior, middle and posterior.

culus, and then runs backwards to join the hind-intestine, about the level of the proximal limb of the ventricular loop. This may be termed the *post-helicoid region* (Text-fig. 1, *27*).

Immediately after the constriction at the upper pole of the proventriculus the alimentary canal widens to form the anterior portion of the thoracic ventriculus, which has a diameter equal to the width of the proventriculus. As it passes towards the abdomen the thoracic ventriculus gradually narrows until its diameter has diminished by nearly a half. After the gut enters the abdomen its diameter again increases up to the middle of the abdominal ventriculus. From this region it gradually decreases to the ventral bend of the ventricular loop, and then again increases. The helicoid region is nearly as wide as the abdominal ventriculus and the post-helicoid region slightly narrower.



Text-fig. 10

In *Musca domestica* and in *Fannia canicularis* the mid-intestine has the same general arrangement as in *Calliphora erythrocephala*, but in *Sarcophaga* it is much less convoluted, the helicoid region consisting of two half-coils connected by an acute bend (see p. 235).

B. Surface markings

While the thoracic ventriculus and, to a much lesser extent, the abdominal ventriculus have a corrugated appearance the surface of the rest of the mid-gut appears smooth.

The external surface of the thoracic ventriculus exhibits a series of rounded, transverse folds with deep narrow depressions between them. This appearance is due to the inner coats bulging outwards in weak, laterally placed, dorso-ventrally elongated spaces where the transverse muscle bands are lacking (Text-fig. 7 B; Pl. VI, figs. 1, 3). Each transverse fold extends from near the mid-dorsal to near the mid-ventral line, but usually, owing to constrictions, appears to consist of three separate portions, a long medio-lateral portion, slightly constricted in the centre, and shorter dorso- and ventro-lateral portions. The constrictions are due to longitudinal muscle bands crossing the folds, and are most marked where several longitudinal bands lie close together. A few longitudinal bands placed a short distance apart in the mid-lateral line together with a fusiform expansion of the transverse bands cause the moderate mid-lateral constrictions. Similar dispositions of the muscle bands cause the more marked dorso- and ventro-lateral constrictions. The peculiar decussations of the transverse muscle bands along the mid-dorsal and mid-ventral lines interrupt the folds and

give rise to longitudinal depressions, and, at least in the anterior region, cause the folds to be disposed alternately on the two sides of the gut (Text-fig. 7 C).

The extent to which the folds protrude depends to some extent on the amount of food present, but under all conditions the folds become gradually less marked towards the distal end of the thoracic ventriculus. In the abdominal ventriculus they are poorly defined and disappear in the region of the ventricular loop.

C. The musculature

The musculature consists of (*a*) outer longitudinal and (*b*) inner transverse or circular bands, which anteriorly are continuous with the muscles covering the proventriculus. There also arises (*c*) from each side of the anterior portion of the thoracic ventriculus a long, thin muscle which passes forward to the thoracic wall to be inserted near the side of the "neck." These muscles consist of fibres derived from several of the longitudinal and some of the transverse muscle bands of the ventriculus. They probably counteract any tendency to displacement of the ventriculus and have been termed the *adductor muscles of the ventriculus* (Text-fig. 7, *a*).

(*a*) *Longitudinal muscles*

Between forty and fifty thin, parallel *longitudinal bands* may usually be counted, each consisting of a few fibres. Usually these bands are separated by considerable intervals, but in eight situations in the circumference of the gut they are placed closer together (Text-fig. 7 B). In the mid-dorsal and mid-ventral lines six to eight bands run moderately close together, covering the decussations of the transverse bands (Pl. VI, fig. 3), while in the mid-lateral lines and also in the lines intermediate between the mid-lateral and mid-dorsal and the mid-lateral and mid-ventral respectively three or four bands run moderately close together causing constrictions in the transverse folds. Sometimes a band divides and is continued as two longitudinal bands (Pl. VI, fig. 3), and occasionally neighbouring bands coalesce to form one band. More rarely a band divides and after a short course the new bands coalesce. Occasionally a small branch passes obliquely from one longitudinal band to another. Very rarely a longitudinal band has been seen to arise as a branch from a transverse band.

Nuclei are found in slight swellings in the bands placed at apparently irregular intervals. No change in the disposition of the longitudinal bands occurs throughout the mid-gut.

(*b*) *Transverse or circular muscles*

While the circular fibres of the vault of the proventriculus form a uniform sheet their continuations on to the *thoracic ventriculus* become separated into bands with spaces between them (Pl. V, fig. 2). At first the spaces are so small that little bulging of the epithelial layer takes place through them and consequently the external surface of the most anterior portion of the ventriculus is almost smooth. Within a short distance, however, the transverse bands assume a very regular disposition. Each flat band, consisting of six to eight fibres, runs in the dorso-ventral line. The fibres composing it run nearly parallel except at the mid-lateral line and two lines intermediate between it and the mid-dorsal and mid-ventral lines, where they become spread out to form slight fusiform expansions. A single nucleus is found in each intermediate fusiform expansion, so that in the long axis of the mid-gut four lines of nuclei belonging to the transverse bands are seen (Pl. VI, figs. 1, 3). The intervening non-muscular spaces are four times as broad as the bands. In the mid-dorsal and mid-ventral lines the bands spread out and unite. The central fibres pass direct from the

band on one side to the band on the opposite side of the gut, but some of the lateral fibres pass laterally not to the directly opposite but to laterally placed bands on the opposite side, and others divide sending branches both to opposite and laterally placed bands. This decussation of fibres is illustrated in Pl. VI, fig. 3. In the proximal region of the ventriculus the bands on the two sides alternate, but later they come to lie nearly opposite to each other. The spaces between the bands gradually diminish in breadth towards the distal end of the thoracic ventriculus.

In the *abdominal ventriculus* the same arrangement is continued, but the bands are relatively close together and there is consequently less bulging of the epithelium through the spaces. The gut, therefore, has a smoother appearance. Decussation of some of the lateral fibres still occurs, but much more obliquely, and is, therefore, less evident, and the fusiform expansions become less marked and finally disappear. From the end of the abdominal ventriculus to the commencement of the hind-gut the spaces between the bands are very narrow, and, though the bands can still be recognised, the muscle seems to have a uniform disposition (Pl. VI, fig. 5). The decussations owing to their great obliquity can scarcely be recognised, but the four lines of nuclei are still evident.

D. THE EPITHELIUM AND DIGESTION

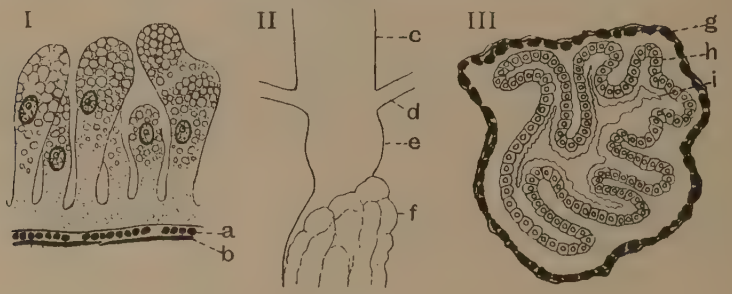
In the mid-gut the epithelial cells have no chitinous intima, and throughout are arranged in a single layer, though crowding together often suggests that several layers are present. In all regions the individual cells vary in height, but the largest are found in the ventriculus and the lowest in the post-helicoid region. Each cell possesses a large oval nucleus, which is usually situated in the middle third of the cell, and the free margin often exhibits a hyaline border. The cells lining the proximal portion stain less deeply with haematoxylin than those lining the distal portion of the mid-gut. Wigglesworth (1929, p. 318) has observed under favourable conditions tracheoles "as pale convoluted threads within the deeply staining cytoplasm."

In some sections, especially when the peritrophic membrane is not greatly distended with food, the condition of the cells in certain areas resembles closely that described in *Tabanus* after a small meal by Patton and Cragg (1913, p. 125). Numerous pale globules of various sizes are present in the protoplasm, especially between the nucleus and the free border, often being so numerous as to distend the cell. Sometimes these coalesce to form large globules. Some of the swollen cells are attached to the basal membrane by narrow stalks (Text-fig. 11 I). Larger and smaller rounded masses of protoplasm containing numerous globules, presumably extruded from the cells, may be present in the lumen of the gut beneath the peritrophic membrane. Rarely free degenerate nuclei are seen in this situation. Similar appearances are described by Wigglesworth (1929, Text-fig. 3 A) in the middle segment of the mid-gut of *Glossina* about one hour after a small meal. On the other hand in some sections of *Calliphora* showing the peritrophic membrane distended with food the cells seem to be flattened out and resemble cubical epithelium.

No detailed observations on the changes which occur in the cells of *Calliphora* during digestion appear to have been published, but Patton and Cragg (1913), after describing the changes which occur in *Tabanus*, state that "in the Muscid flies one finds, in most sections, cells at all stages of this process, whether the gut is distended with blood or not; a row of normal resting cells is seldom seen. The conditions found in the biting flies are approximately the same as in *Musca*, although their feeding habits are entirely different, as they take one large meal and then rest while digestion proceeds, while *Musca* and its allies feed intermittently." Usually, when certain materials, such as semi-liquid faeces, are obtainable,

however, these flies ingest food until, after a few minutes, the alimentary canal from the proventriculus to the anus, and sometimes the crop, are completely filled (Pl. IX, fig. I), and moreover "if a fly has access to faeces it will feed every few minutes and as often evacuate its intestinal contents" (Wenyon and O'Connor, 1917). Much of this material apparently passes unchanged through the gut for Wenyon and O'Connor showed that flies allowed to feed on liquid faeces containing active *Trichomonas* passed moist droplets within five minutes containing "living unaltered *Trichomonas*."

Wigglesworth (1929, p. 295), who has studied the enzymes in the alimentary canal of *Glossina* and of *Calliphora*, finds that in *Calliphora* amylase is present in large quantities in the salivary glands, amylase (+ + +), invertase (+ +) and maltase (+) in the ventriculus and the same three enzymes in larger quantities together with tryptase and peptidase in smaller quantities in the helicoid and post-helicoid regions. Lactase and peptase are absent. On the other hand none of the enzymes investigated were found in the salivary glands of *Glossina*, and only tryptase in small amount in the anterior segment of the mid-gut. In the middle segment amylase (+), tryptase (+ + +) and peptidase (+ +) were found and in the posterior segment tryptase only.



Text-fig. 11

"The results with *Calliphora*, which is rich in enzymes acting upon carbohydrates and very poor in proteolytic enzymes, afford an interesting contrast. Here again it is to be noted that the enzymes are most active in the hinder part of the mid-gut."

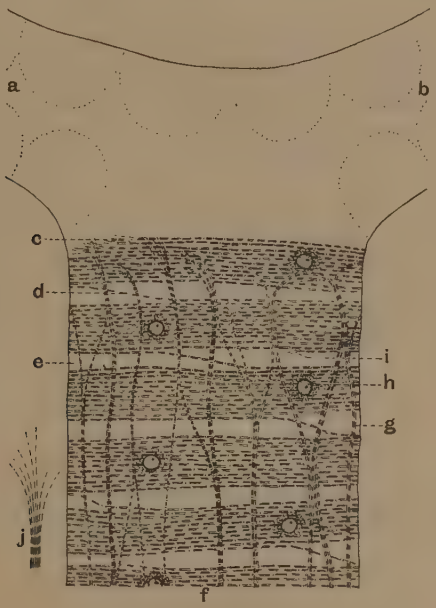
Human blood ingested by *Calliphora* is found apparently unaltered in the rectal pouch.

VIII. THE MUSCULATURE AND MOVEMENTS OF THE MALPIGHIAN TUBES

The four Malpighian tubules are very long and difficult to unravel completely, but they measure not less than 40–45 mm. in length. The outer basement membrane supports large cells of a yellow colour, containing pigment and oil globules, whose bases bulge outwards and give the tubule a beaded appearance. The lumen is intercellular. The two Malpighian tubules on each side unite and immediately open into a pale, smooth-walled, common duct.

The two common ducts, each of which is about 2 mm. in length, open into the terminal portion of the mid-gut in the right and left mid-lateral lines respectively, immediately above the anterior limit of the chitinous intima of the hind-gut. The epithelium lining the common ducts is similar to that lining the mid-gut. The basement membrane of the tubules and of the ducts is covered by a very close and extensive network of tracheoles.

Eastham (1925), working with all stages of *Drosophila funebris* and *Calliphora erythrocephala*, was apparently the first to show that in Diptera the common ducts are covered externally by longitudinal and circular muscle fibres, but he does not describe the musculature in the adult *C. erythrocephala*.



Text-fig. 12

In *C. erythrocephala* there are from eight to twenty *longitudinal muscle bands*, which arise from neighbouring longitudinal bands of the mid-gut (see p. 206). In their course they divide and coalesce in the same manner as the corresponding bands in the mid-gut. These bands continue up to the distal end of the common duct, where, over the last band of circular muscle, some of them divide, the branches either passing laterally to mingle with the fibres of the circular band, or forming loops coalescing with neighbouring longitudinal bands, or breaking up into fans of fibrils, which are apparently inserted into the basement membrane. Other bands without dividing either end in fans of fibrils or bend laterally to mingle with the circular band (Text-fig. 12).

The inner *circular muscle* is disposed in definite broad bands, consisting of ten to twenty fibres, with one large nucleus in connection with each band. These nuclei are arranged in two somewhat irregular lines, each line being mainly composed of the nuclei of alternate bands. The bands are connected by occasional fibres passing diagonally across the spaces

separating the bands. Rarely a fibre breaks up into a fan of fibrils, which seem to be inserted into the basement membrane. This presumably represents either the origin or the termination of a fibre. At the distal end of the common duct the musculature terminates abruptly at the margin of the last circular band, the distal fibres of which break up into fans of fibrils apparently inserted into the basement membrane (Pl. VI, fig. 2).

In the honey-bee (*Apis mellifica* L.) Trappmann (1923) described muscle fibres passing round the tubules of the worker, but Morison (1928, p. 585) states that "muscle fibres are present on the Malpighian tubes of all castes...the fibres vary from one to four per tube, around which they are twisted in a spiral which circles the tube about three times." Eastham (1925) was unable to find any spiral fibres on the tubules of *Drosophila*, and no such fibres have been found on the tubules of *Calliphora erythrocephala*.

Eastham (1925, p. 393) observed that "the Malpighian tubules of all stages in the life history of *Drosophila funebris* and of *Calliphora erythrocephala*...are capable of movement and that peristalsis does occur...in the short common tubules proximal to the gut...undoubtedly due to the rhythmical contractions of the striated muscle fibres found there." In regard to the movements of the tubules he states that "if the tracheae connecting the tubules to the alimentary canal are carefully dissected away, the tubules still seem to wave about in the salt solution." The author has frequently observed peristaltic movements in the common ducts and contortions of the tubules in flies opened under salt solution with as little disturbance as possible, and also movements in detached portions of tubules. In intact tubules flowing movements of particles towards the ducts, which Eastham thinks are induced by peristalsis in the ducts, were observed on several occasions.

IX. THE HIND-GUT

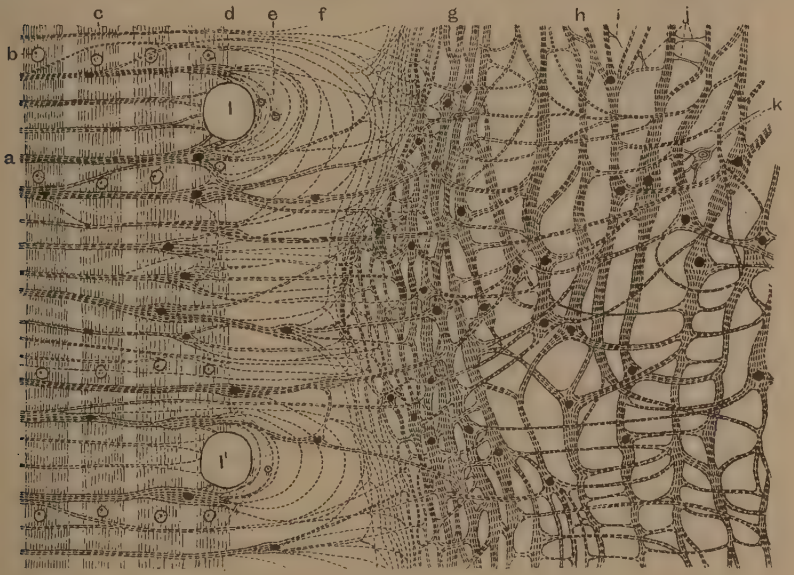
A. Definition and anatomy

Lowne (1892, p. 411) includes under the term hind-gut or metenteron "that part of the intestine which intervenes between the ducts of the Malpighian tubes and the rectal valve." Hewitt (1907, 1914) uses the term in the same sense, but Giles (1906) and Patton and Cragg (1913, p. 116) include the whole region between the Malpighian tubes and the rectal pouch. In this paper Lowne's definition is employed and the rectal valve and the region following it are considered separately.

The hind-gut from its commencement runs forwards ventral to the mid-gut almost up to the anterior end of the abdomen, where it bends, and returning nearly to its place of origin becomes modified to form the rectal valve (Text-figs. 1 and 10). It is usually somewhat smaller in diameter than the mid-gut and has a less delicate appearance.

It is lined throughout by a chitinous intima, which commences as a thin membrane at a very definite line encircling the gut just distal to the orifices of the Malpighian ducts (Text-fig. 13, *d*), and becomes stronger distally, especially in the proximity of the rectal valve. The intima lies on and is adherent to a single layer of cells. Except in the very short proximal region, distinguished by its transitional musculature (Text-figs. 11 II, and 13, *f*), where the cells are small and low, this epithelium consists of regular, cubical, deeply staining

cells with rounded nuclei, interrupted occasionally in the distal region by small groups of larger, more rounded cells with vacuolated protoplasm. The epithelium rests on a strong basement membrane. External to the basement membrane lies the muscle coat, but this is not so intimately related to the basement membrane as it seems to be in most other regions of the gut, though it is not easy to separate the two by dissection. Long, very fine strands passing from the muscle sheaths to the basement membrane connect the two structures, and tracheae, passing between the muscle bands to reach the basement membrane, also assist in connecting them. When the hind-gut is full of food the inner surface is smooth and circular, but when it is empty the inner coats are thrown into several, usually six, strong longitudinal folds. The muscle coat, though showing some longitudinal folding, does not, owing to its loose attachment, follow the deep infolding of the inner coats (Text-fig. 11 III).



Text-fig. 13

B. MUSCULATURE OF THE HIND-GUT

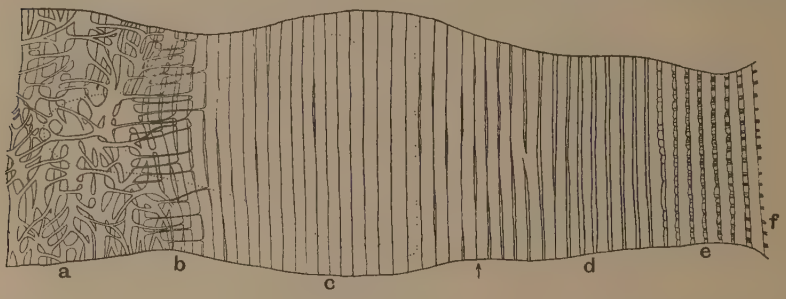
At the distal end of the mid-gut its circular muscle bands cease suddenly, the distal margin of the last band being at the level of the centres of the orifices of the Malpighian ducts, though some fibres of the circular coat, supplemented by branches from the longitudinal bands, pass immediately distal to the orifices. Above this level the nuclei of the circular bands, as elsewhere in the mid-gut, are arranged in four lines, but below this level two or three nuclei of the series are found in connection with the fibres passing distal to the orifices.

External to the distal circular bands many of the longitudinal bands of the mid-gut develop nucleated stellate expansions, which terminate in fans of longitudinal, or slightly divergent, oblique branches, some of moderate dimensions, but mostly small. These branches pass on to the hind-gut and for a short distance constitute the only musculature, there being no transversely disposed bands, except occasional, short, transverse fibres, uniting adjacent branches (Text-fig. 13, *f*). This transitional region is recognisable in a fresh whole prepara-

tion as a smooth area immediately distal to the Malpighian tubes (Text-fig. 11 II). At different levels most of these branches ultimately divide, and the new branches, which tend to run dorsally and ventrally, give rise to several transverse bands. A few of the more oblique branches join the transverse bands without dividing. The most proximal of the newly formed transverse bands are thin, but very shortly a series of definite, somewhat closely disposed, broad transverse bands are formed, which seem to act as a sphincter. The line of this sphincter is uneven proximally, since between the ducts on the ventral side characteristic transverse bands are developed farther forward than elsewhere. The bands forming the sphincter have the same characteristics as those of the remainder of the hind-gut, but are crowded more closely together.

The few longitudinal bands of the mid-gut which are in a line with the orifices of the Malpighian ducts each break up into two or three smaller bands, which, together with lateral branches from neighbouring longitudinal bands, pass on to the ducts to form their longitudinal bands. Longitudinal bands passing lateral to the ducts break up into fans of branches, some of which form loops distal to the ducts, while others pass on in the ordinary manner to give rise to transverse bands of the hind-gut.

The musculature of the whole of the hind-gut from the sphincter to the region of the rectal valve has the following disposition. Broad bands with distinct intervals between



Text-fig. 14.

them pass transversely, most of them commencing by the fusion of small oblique bands and terminating in nucleated, stellate expansions (Text-fig. 13, *h*; Pl. VI, figs. 6, 7). Branches of various sizes arise from the transverse bands both at irregular intervals throughout their length and from the terminal expansions. Nuclei are often found near the origins of the larger branches. The branches pass longitudinally or somewhat obliquely to unite either with adjacent or more distant transverse bands or sometimes with branches of other bands. They usually pass external to any transverse bands which they cross. Communications between the muscle sheaths transmitting no muscle fibres, like those described in the musculature of the crop (p. 184), occur at frequent intervals, especially between the transverse bands (Text-fig. 13, *j*). This musculature, therefore, consists of a complex network, with the larger transverse bands situated internally to the smaller longitudinal branches. There is no separate longitudinal system of muscles.

In the neighbourhood of the proximal end of the rectal valve the transverse bands seem to become more irregular and their lateral branches more numerous (Text-fig. 14, *a*). Within a short transitional region (*b*), however, the transverse bands rapidly become more closely approximated and less branched, and at the same time the branches become almost wholly longitudinal and finally cease. Beyond this transition area is the rectal valve region (*c*). Here the musculature consists of broad, regular, non-branching, closely approximated transverse bands. The rectal valve region is followed by the first part of the rectum (*d*), the

musculature of which consists for the most part of similar transverse bands, which are not so closely approximated, except at both ends, where the bands are closely applied and seem to form sphincters. In both these regions there are very numerous communications between the muscle sheaths, but these transmit few, if any, longitudinal branches. At the distal end of the first part of the rectum (*e*) fine longitudinal branches arise, at first in small and later in greater numbers. These run distally, internal to the transverse bands, and by uniting in groups form the longitudinal, inner bands (*f*) of the musculature of the pouch.

Peristaltic waves have frequently been seen to commence a little below the orifices of the Malpighian tubes and pass down the hind-gut as far as the commencement of the rectal valve region, but, when the valve is in the closed position, not beyond that point.

X. THE REGION OF THE RECTAL VALVE

A. INTRODUCTION

The term "region of the rectal valve" is here applied to the short length of gut, measuring about 0·15 mm. in length, which is distinguished externally from the preceding part of the hind-gut partly by its smooth appearance, due to the strictly circular arrangement of the fibres of its muscular layer, and especially by the presence of a well-marked swelling encircling the gut, usually situated a little distal to the centre of the smooth portion (Text-fig. 1, *17*). Internally this region is lined by thickened yellowish intima from which spines project, except in one area, into the lumen.

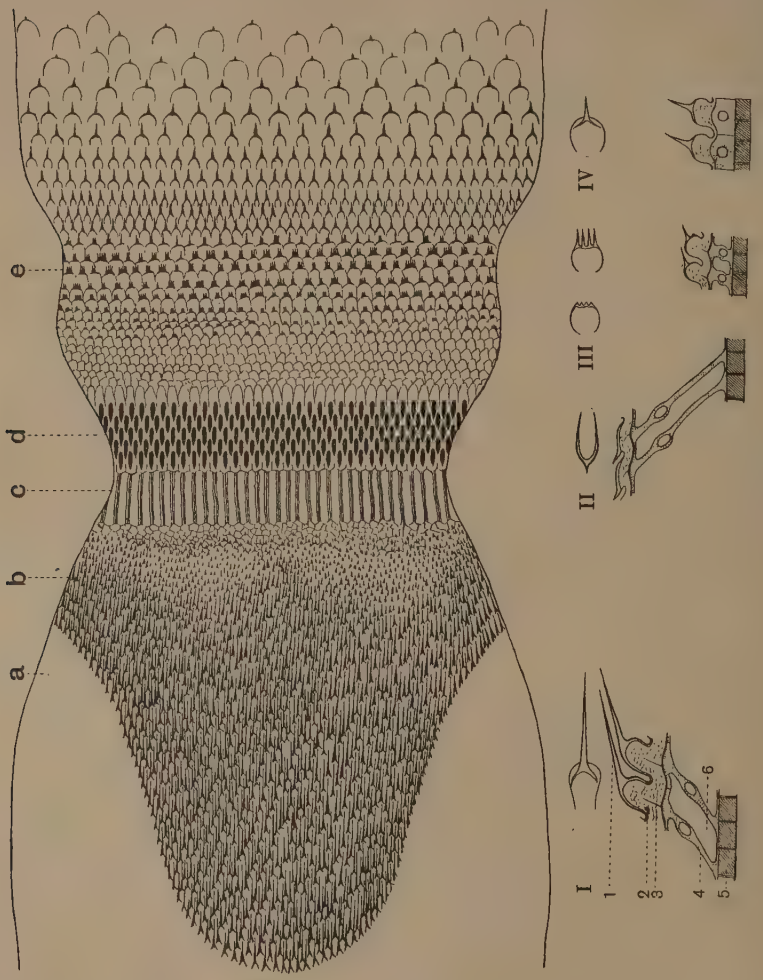
Lowne (1895, p. 411) gives a short description of this region, stating "that the lower part of the distal intestine and the valve which separates it from the rectum are lined by chitinised cells which have spines projecting into the lumen of the gut." He considers "it is possible that the remains of the food are subjected to trituration as they pass through the distal portion of the distal intestine and the rectal valve." Apart from this short description he gives a diagrammatic illustration of the valve in longitudinal section, but without any explanatory text.

The valve is not mentioned by Hewitt (1907), Patton and Cragg (1913), Patton and Evans (1929) or by most other authors.

General description. In the neighbourhood of the proximal end of the valve region the complex muscle fibres of the hind-gut (Text-fig. 14, *b*) become arranged transversely, and over nearly the whole length of the region the musculature consists of strong circular fibres, without any trace of longitudinal fibres.

On opening the gut longitudinally in this region it is seen that four portions can be recognised readily: (1) a wide *proximal portion* with long, distally directed spines, (2) a short, non-spinous, *longitudinally striated portion*, (3) a short portion bearing strong, flat and, in the open position of the valve, proximally directed spines, which are so closely apposed as to form a pavement—the *pavement portion*, and (4) a *distal portion* with short, distally directed spines (Text-figs. 15 and 16 IX).

While in such preparations the proximal and distal portions lie horizontally, the longitudinally striated and pavement portions form a considerable projection into the lumen, their adjacent margins meeting at an angle (Text-fig. 16 IX). These two portions constitute the rectal valve of Lowne. It is



Text-fig. 15

evident that in the region of the valve the intima exceeds the muscle layer in length, and under natural conditions has to arrange itself in the form of an inwardly projecting fold. In the natural condition the muscle covering the fold is bulged outward in order to accommodate it and forms the swelling

encircling the gut. When, however, the gut is opened longitudinally, this swelling almost disappears.

On the cut surface there is seen underlying the strongly chitinised internal layer of the intima a gelatinous opalescent layer, which is very thin at both ends of the region, but increases in thickness towards the centre, attaining its maximum beneath the longitudinally striated portion. This layer consists of soft chitin, and may be termed the *opalescent layer* of the intima (Text-fig. 16 I, *j*, *n*; IX, *j*).

The cells underlying the opalescent layer of the intima are cubical at either end of the region, but become progressively elongated in passing centrally, until below the junction of the second and third portions they form a compact mass of long fibres projecting inwards like a *shelf* to occupy a V-shaped depression in the opalescent layer, caused by the angle to each other at which these layers are disposed (Text-fig. 16, *f*). Externally the basement membrane, on which these cells lie, is adherent to the sheath of the muscular layer (Text-fig. 15 I, II, III, IV). This arrangement allows of a limited degree of movement of the central part of the intima in relation to the muscular coat, a movement which results in the opening and closing of the valve (see p. 212).

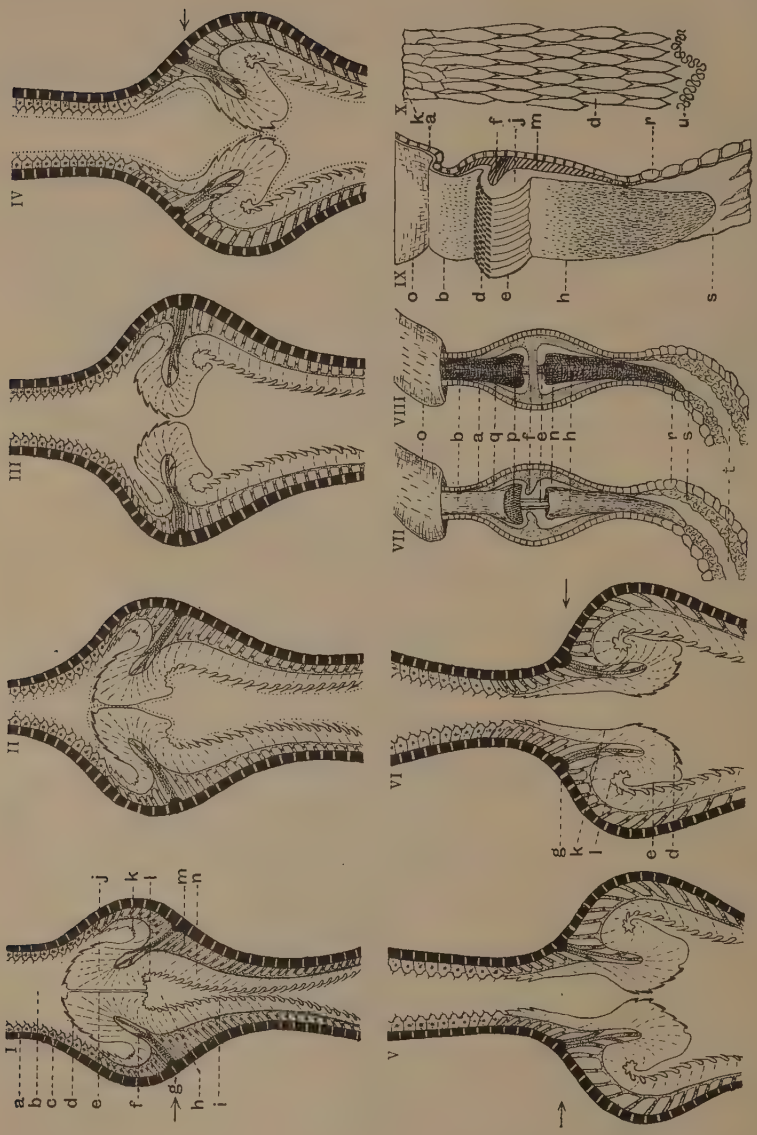
B. Description of special portions

(*a*) *The proximal portion*

Internally the proximal portion commences in a longitudinal yellowish thickening of the intima, situated on the concave side of a distinct and constant curve in the gut (Text-fig. 16 VII). This thickening broadens as it passes distally till its margins meet and it surrounds the lumen (Text-fig. 15, *b*). The portion surrounding the lumen is continued distally for a short distance, at first slightly increasing in diameter, and then narrowing in order to unite with the proximal margin of the striated region.

Proximally the thickened intima is provided with long, sharp, distally directed spines (Text-fig. 15, *b*), arising from irregular, quadrilateral plates, overlying proximally cubical and distally elongated cells. The spines soon increase in size, and possess very long tapering points (Pl. VII, fig. 5). About the centre of the portion surrounding the lumen the spines begin to diminish in size and in number, and soon become short and irregular in disposition. Near the junction of this portion with the striated portion the spines cease, but the thick chitin still shows divisions into irregular plates, some of which close to the junction are elongated. A short distance beyond the proximal margin of this portion the opalescent layer commences and gradually becomes thicker up to its distal margin (Text-fig. 16, *n*). This layer separates the strong, yellow, internal chitin from the underlying cells. It is soft, and has a semi-transparent gelatinous appearance, and is traversed by fine fibrillae passing transversely through its substance, apparently from the cells to the corresponding internal plates bearing the spines (Text-fig. 15 I, *3*). Fixation usually causes great shrinkage in this layer, which is best seen in whole fresh preparation (Text-fig. 16 VII) or in dissections (Text-fig. 16 IX). In stained preparations the fibrillae take the stain more intensely than other parts of the opalescent layer.

The cells external to the opalescent layer gradually elongate from the cubical cells of the proximal region into very elongated cells, having the appearance of fibres, passing between the base of the opalescent layer and the muscle sheath (Text-fig. 15 I, *4*).



Text-fig. 16

The broadest portions of these cells underlie the opalescent layer. The space surrounding these cells presumably contains haemocoele fluid (Text-fig. 15 I, *6*). Apart from other functions these cells act as strands limiting excessive movements of the intima during the opening and closing of the valve.

(*b*) *The longitudinally striated portion*

This portion is lined by intima devoid of spines or well-defined plates, but thrown into a number of deep longitudinal folds (Text-fig. 15, *c*), with numerous subsidiary folds (Text-fig. 16 X, *u*). In the closed position of the valve the walls of this portion are in apposition (Text-fig. 16 I), save for the intervention of the thin, empty peritrophic membrane, and the lumen of the gut is represented by a star-shaped slit. During the opening of the valve the walls separate and the folds in them are widened (Text-fig. 16 II–V) and finally complete evagination occurs, so that the part of the intima originally facing inwards then faces outwards (Text-fig. 16 VI).

The opalescent layer underlying this region is very thick and shows well-marked fibrillae. The underlying cells are long, especially those towards its distal end, which take part in the formation of the shelf.

In nearly all positions assumed by the valve this portion unites with the succeeding pavement portion at an acute angle, and consequently a deep depression seems to be formed in the opalescent layer (Text-fig. 17 II, *d*), which is occupied by elongated cells attached to the muscle sheath along a narrow band. In section these cells give the appearance of a shelf projecting into the apparent depression in the opalescent layer (Text-fig. 16 I–VII), and, when the intima is removed in slightly macerated, longitudinally opened preparations, it often happens that most of these cells lose their connection with the intima and remain attached to the muscular layer as an inwardly projecting shelf (Text-fig. 17 I, *h*).

(*c*) *The pavement portion*

In the closed position of the valve this portion is reflected outwards and forwards on all sides from its union with the margin of the slit-like orifice of the striated portion, giving rise to the appearance of a dome, pierced centrally by the striated portion (Text-figs. 16 VII and 17 II, *c*). The outer margin of the dome is reflected towards the rectum to unite with the succeeding distal portion.

During the opening of the valve this portion becomes invaginated and finally forms the inner wall of the valve (Text-fig. 16 VI).

In this region five rows of modified spines occur. Each spine consists of a strong, broad, nearly parallel-sided, elongated ridge, arising from a quadrilateral plate of the intima. Only its proximal end, which is produced into a short strong spicule, is free (Text-fig. 15 II). In the closed position of the valve the free ends of the spines are directed distally, but in the open position, owing to the invagination, they are directed proximally, namely in the opposite direction to the spines of the proximal and distal regions.

These spines at all times lie very close together and therefore provide this portion with an internal surface like a pavement or the pharyngeal plates of a skate (Text-fig. 16 X).

The opalescent layer underlying this portion is somewhat thinner than that underlying the preceding portion and forms the distal wall of the apparent depression. The fibrillae are also less numerous. The cells in connection with this portion are mostly very elongated and form the distal part of the shelf (Text-fig. 15 II).

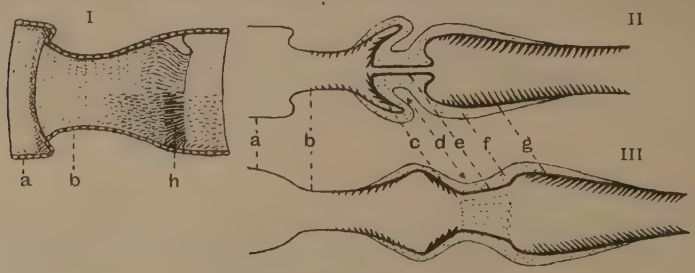
(*d*) *The distal portion*

The distal portion extends from the distal margin of the pavement portion to the commencement of the first part of the rectum. Its lumen is wide, and is lined by a strong chitinous intima. Proximally the intima exhibits no spines, but its surface is divided into

somewhat irregular plates. Proceeding distally these plates exhibit minute indentations on their distal margins, and these gradually increase in size till the plates are armed with long, very thin spines. Some plates have only a single spine, but most exhibit a comb-like row of two to five spines, closely resembling those found on the rectal papillae (p. 221). More distally one spine of the row elongates and the others diminish, so that the plates, which increase in size, come to exhibit single, moderate-sized, strong spines. Near the distal end of the region the spines again become smaller and almost disappear (Text-fig. 15, *e*).

The opalescent layer is clearly seen at the proximal end of this region, but it gradually diminishes in thickness, and becomes unrecognisable towards the centre of the region (Text-fig. 16 VII, IX). Similarly the cells underlying the opalescent layer diminish in length, and become cubical distally.

The structure of the inner surface of the intima is best seen by dissecting the intima from the muscle in specimens macerated by immersion in salt solution for a few hours. The opalescent layer remains attached to the strong internal chitin and can be well seen, while many of the cells usually remain attached to the muscle. The elongated cells are well exhibited in frozen sections, cut without previous fixation. Fresh whole preparations, stained for 30 min. in salt solution containing 0·05 per cent. potash and a little picro-carmine and mounted under a cover-glass in dilute potash solution, often show remarkably well in optical section the cells and the opalescent layer with its fibrillae. So much shrinkage of



Text-fig. 17

the opalescent layer and crowding of the elongated cells usually occurs in paraffin sections that interpretation is difficult, unless other methods have been resorted to previously.

The study of fresh preparations in warm salt solution under a binocular dissecting microscope gives interesting information. With transmitted light it is possible to define with some accuracy the positions of the various portions, including the limits of the almost transparent opalescent layer and of the shelf and other areas occupied by the elongated cells (Text-fig. 16 VII). With a dark background the chitinous structures exhibit little differentiation, but the shelf is remarkably well shown as a grey band, apparently dividing the clear opalescent area into two parts (Text-fig. 16 VIII).

If longitudinal traction is made on a fresh preparation the muscle often ruptures in the neighbourhood of the valve and the intima (including the opalescent layer) is drawn out as a tube which is narrowest at the proximal end of the pavement portion. When released the intima tends to return towards the form it assumes when the valve is in the closed position (Text-fig. 17 II, III).

(*e*) *The mode of action of the rectal valve*

Movements leading up to the opening and closing of the valve can be seen in fresh preparations, but usually they are very rapid. Occasionally, however, the movements are slow and then it is possible not only to follow the changes

occurring in the valve, but to fix examples in different positions (Pl. VII, figs. 2, 3).

The opening of the valve coincides with and seems to be brought about by a wave of reverse peristalsis, passing from the distal to the proximal part of the valve region. This wave seems to be preceded by a wave of relaxation.

As the wave of constriction passes along it presses on the dome-shaped intussusception causing invagination of the pavement intima, which ultimately faces inwards instead of outwards, and evagination of the striated portion, which ultimately faces outwards instead of inwards. This reversal is brought about because the area of attachment to the muscle sheath of the outer ends of the elongated cells of the shelf remains throughout approximately in the same position (Text-fig. 16, arrows), while the internal ends of the cells are free to move through an arc of nearly 180°. The internal ends are attached to the centre of the valve and limit its movements within that range. The elongated cells underlying other parts of the opalescent layer are capable of similar movements and limit, according to their lengths, the excursions of the parts of the intima to which they are attached. At both ends of the valve region the cells are so short that the intima is closely bound to the muscle and little movement is possible. There is no evidence that these cells are contractile.

Some of the positions assumed by these parts when passing from the closed to the open position of the valve are illustrated diagrammatically in Text-fig. 16 I–VI.

The valve usually remains open for a very brief period only, during which the intestinal contents are forced through by contractions in the distal part of the hind-gut. Then a wave of contraction passing towards the rectum restores the valve to the closed position, which is maintained by tonic contraction of the fibres surrounding the striated region.

(*f*) *The functions of the rectal valve*

(1) The circular muscle bands immediately proximal and distal to the valve seem to act as sphincters, preventing, except when the valve is in the open position, the passage of the contents of the hind-gut forwards or of the contents of the rectum backwards to the valve itself. It is common to observe waves of contraction passing downwards in the hind-gut and ceasing in the neighbourhood of the valve without causing any forward movement of the contents through the valve. Also very violent movements of the walls of the first part and pouch of the rectum are common, especially when these portions of the gut are greatly distended, but their contents have never been seen passing backwards into the hind-gut.

In the fully closed position of the valve the inner walls of the striated region are in apposition except for the interposition of the empty peritrophic membrane which lies in the lumen (Pl. VIII, fig. 6).

(2) The valve itself at appropriate times allows of the passage of some of the contents of the hind-gut into the rectum.

(3) It is possible that undigested objects may be crushed in their passage through the valve, for while the pavement portion is being evaginated in order to resume the closed position a crushing action of considerable intensity must be exerted on any materials becoming engaged in the rolling pavement of hard ridge-like plates.

In flies fed on banana large masses up to $2 \times 0{\cdot}3$ mm., which must shortly pass through the valve, may occasionally be observed in the hind-gut, and possibly hard substances occasionally reach this region.

(4) Short lengths of peritrophic membrane are dragged through towards the rectum during each action of the valve.

This appears to be the chief function of the spines. The spines of the proximal portion indent, and possibly penetrate, the peritrophic membrane in their neighbourhood, preventing it from slipping back into the hind-gut when the reverse peristaltic wave occurs, and perhaps they aid in pushing it forward when the downward wave of constriction leading to closure occurs (Pl. VII, fig. 1). During their rotation from the closed to the open position of the valve the spines of the pavement portion probably fail to engage in the peritrophic membrane because it is then not distended, but in their rotation from the open to the closed position they probably indent the membrane, which is then distended with intestinal contents, and pull it onwards. The spines of the distal portion apparently serve to retain in position the portion of the membrane which has passed through the valve, especially when the wave of reverse peristalsis leading to the next opening occurs.

Whether any of the various spines which indent the peritrophic membrane cause minute perforations in it has not been definitely ascertained, but it is clear that no large rents are caused, for the membrane can be traced into the rectum apparently intact and distended with food material. Also in stained preparations of portions of the membrane extracted from the first part of the rectum no perforations have been seen.

In *Protocalliphora* (Text-fig. 16 VII), *Lucilia*, *Pollenia*, *Musca* and *Fannia* the valves and their surroundings are essentially similar in structure.

XI. THE RECTUM

Lowne (1895, p. 412) describes the rectum as consisting of three parts, (A) a proximal *first part*, dilated into a small sac, in which he thinks the residuum of nutritive matter after trituration in the rectal valve may be absorbed, (B) a dilated second part—the *rectal pouch*—into which the rectal papillae project, and (C) the third or *anal part*, which is tubular and ends at the anus.

A. The first part of the rectum

The *first part of the rectum* widens rapidly from its junction with the smooth, narrow distal portion of the valve region, and shortly attains a diameter nearly twice that of the latter region. At the point of junction a loose intus-

susception is often formed by the broader first part of the rectum folding over the terminal part of the valve region (Text-fig. 16 VII, *o*; Pl. VII, fig. 2). The rest of the first part of the rectum, except for a slight constriction at its point of junction with the pouch, is a tube of nearly uniform diameter. Its length is slightly greater than that of the valve region.

It is lined internally by a thin, spineless, chitinous intima, covering a cubical epithelium, set on a thin basement membrane. The muscular coat in the proximal three-fourths consists of circular bands, but in the distal fourth longitudinal branches arise from the circular bands and pass distally into the rectal pouch to form its inner longitudinal coat (Text-figs. 14, 18; Pl. VII, fig. 7). Near its junctions with the valve region and the pouch respectively the circular coat is slightly thickened to form sphincters.

When the viscus is empty the basement membrane, cells and intima are thrown into longitudinal folds, up to twelve in number, but the muscular coat is only slightly folded. When the viscus is distended with food these folds are obliterated.

The length of the first part of the rectum relatively to that of the hind-gut differs greatly in different species of flies (see p. 235).

In fresh preparations observed in salt solution very energetic peristaltic contractions are often seen, usually related to contractions taking place in the pouch. Not infrequently, however, though movements occur throughout its length, the contents of the viscus are isolated by tonic contractions of the sphincters.

This part of the rectum, apart from differences in the musculature, resembles the hind-gut.

B. The second part of the rectum or rectal pouch

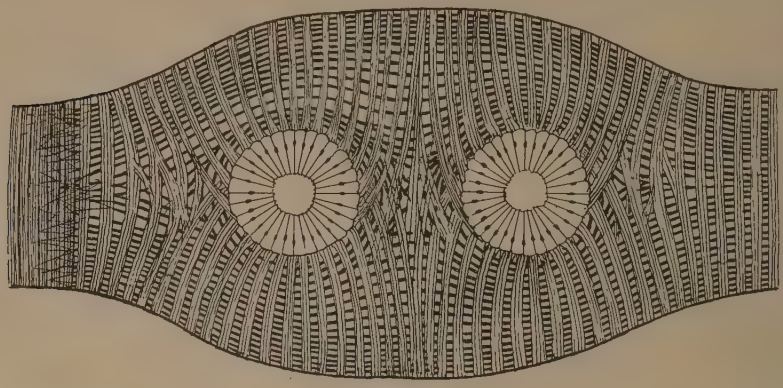
This region is flask-shaped, capable of great dilatation and characterised by the projection into its cavity of four conspicuous rectal papillae, two on each side.

The pouch is lined by flattened cells, covered internally by a thin spineless *intima*. From the muscle sheath, strands, best seen by gently stretching thick frozen sections, pass to the basement membrane. When the viscus is distended the intima forms a smooth internal lining, but when it is contracted the intima together with the underlying cells is thrown into intricate folds, which nearly fill the cavity.

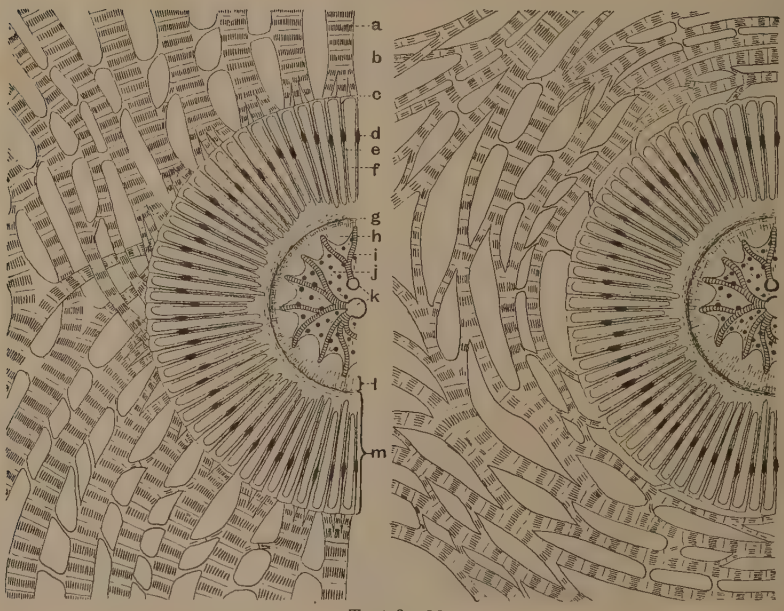
The *muscular coat* consists of two layers, an outer circular, continuous with the coat of the first part, and an inner longitudinal commencing in the distal region of the first part. In the portions of the pouch unrelated to the papillae these layers cross each other at right angles, but near the bases of the papillae both are modified.

On the dorsal and ventral surfaces of the pouch the fibres of the *external muscle coat* are arranged in broad, parallel, transverse bands, but on the sides of the pouch many of them are interrupted and inserted into the radial membranes covering the bases of the papillae in such a manner as to produce radial traction. For this purpose bands which should pass some distance lateral to a papilla are inclined towards its base and give off branches, passing towards it, of which some lie almost in the long axis of the pouch before reaching their insertions. Some of the branches passing ventrally overlap others passing dorsally close to their insertions (Text-figs. 18, 19). Bands more closely related to the papilla do not branch, but are more or less inclined towards its centre. Progressively less inclination

is observed in bands lying closer to the centre of the base until those passing directly towards the centre show no inclination (Pl. VII, figs. 7, 8). Near their insertions most of the bands become slightly broadened and subdivided (Text-fig. 19). The bands from the dorsal surface which pass into the median portion of the space between the two papillae on each side



Text-fig. 18



Text-fig. 19

break up on approaching the level of the bases into broad branches which unite with similar branches of bands from the ventral surface (Text-fig. 18). In the proximal and distal regions of the pouch uninterrupted circular bands are present (Text-fig. 18). Lateral communications between the sheaths, sometimes transmitting fibres, are common (Text-fig. 19).

It is evident that radial traction, which probably serves to keep open during contraction the semilunar foramina and the infundibular space (p. 227), is a matter of great importance.

The *internal muscle coat* commences near the distal end of the first part of the rectum in fine, lateral, distally directed branches from the circular coat, which unite to form parallel longitudinal bands, narrower than those of the external coat. These bands mainly pass either dorsally or ventrally to the bases of the papillae, and on reaching the anal part form its inner longitudinal coat. The bands situated laterally, however, break up into branches, which re-unite to form bands which encircle the bases of the papillae (Text-figs. 18, 19). The sheaths of the bands, more especially around the papillary bases, have lateral communications, like those found in the crop muscles, and in some of these communications fibres pass from one band to another. Fibres also pass from the nearest encircling bands to the radial membranes, and possibly aid in producing traction on these membranes.

Observations on fresh preparations show that uniform peristaltic contractions of the pouch muscles are uncommon, but that violent and seemingly irregular contractions in different regions of the pouch occur almost ceaselessly in well-fed flies, causing the papillae to sway rapidly in all directions and to churn up the fluid contents of the pouch.

Functions of the pouch muscles. Though the pouch muscles do not directly constrict or dilate the papillae they are probably indispensable factors in the proper functioning of these organs. The traction exerted on the radial membranes (1) keeps open the semilunar foramina leading from the abdominal cavity into the infundibular space and intercellular sinus and (2) may, possibly in conjunction with the apparently contractile radial fibres lying in the folds of the radial membrane, exert pressure on the cellular layer thus stimulating movements of fluid in the various spaces within the papilla. The swaying movements of the papilla, brought about by the apparently irregular muscular contractions, possibly cause, as suggested by Engel (1924), the comb-like teeth of the external sheath to perforate or even tear the peritrophic membrane and liberate its contents. At the same time the churning motion causes uniform distribution of the soluble and insoluble constituents of the pouch contents. At appropriate times the pouch muscles force portions of the contents into the anal part of the rectum.

The Rectal Papillae

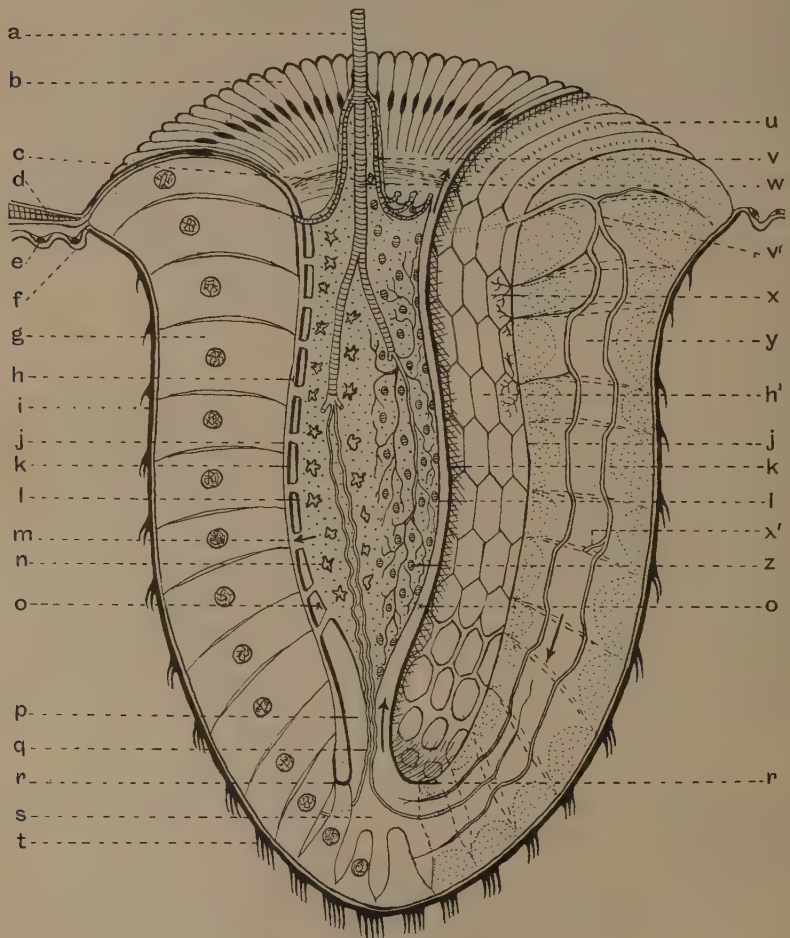
These are four laterally situated, conical bodies, about 0·75 mm. in length, which project into the lumen of the pouch. Two are situated on each side.

They were early described in various insects by Swammerdam (1669) and Treviranus (1817). In the blow-fly they have been described by Ramdohr (1811), Leydig (1857), Lowne (1869) and Chun (1876), and more recently by Cognetti de Martiis (1924) and Borri (1925). These and other workers have made numerous suggestions as to their functions.

In consequence of the different views which have been expressed both in regard to their anatomy and function particular attention has been devoted to them, and it has been found necessary to give names to the structures found in them.

(*a*) *General description*

Each papilla exhibits a round base, embedded in the wall of the pouch. The base interrupts the musculature (see Text-figs. 18, 19) and projects slightly into the abdominal cavity beyond the general line of the wall. The rest of the



Text-fig. 20

papilla, in the form of a gently curved opaque cone with its apex pointing towards the anal part of the rectum, lies within the pouch (Text-fig. 24).

The papilla is a complex organ consisting of several cone-shaped layers (Text-fig. 20). It is probably composed of the structures forming the wall of the pouch, invaginated into it and modified.

Its chitinous *external sheath* is continuous with and represents the intima of the pouch. Within this lies the *cellular layer*, composed of very large cells, apparently continuous with the sub-intimal cells. Internally these cells seem to be bounded by a very thin membrane, the *subcellular membrane*, which may represent the basement membrane or possibly the inner layer of the muscle sheath. The muscle layers of the pouch terminate at the margin of the base, but may be represented over the base by the elongated *radial fibres*, resembling in some respects mammalian smooth muscle cells. The muscle layers are, however, also inserted into the *radial membrane*, which perhaps represents the thickened outer layer of the muscle sheath. The radial membrane covers the base, and near its centre dips sharply downwards, parallel to the inner surface of the cellular layer, to enclose a funnel-shaped cavity, the *infundibular cavity*. This downward continuation of the radial membrane has, therefore, been termed the *infundibular membrane*. Near the apex of the papilla this membrane is reflected on to the inner surface of the cellular layer and seems to become continuous with the distal portion of the subcellular membrane. The infundibular and cellular membranes are usually in apposition, but enclose between them a potential space termed the *subcellular space*. Near the distal end of the papilla the infundibular cavity becomes continuous with a large cavity ramifying between the cells, the *intercellular sinus*. Within the upper two-thirds of the infundibular cavity lies the conical *medulla*. It consists of a limiting membrane, the *medullary sheath*, enclosing a space, the medullary space, into which pass a number of tracheae. Two sets of the tracheae, the cortical and the apical, proceed beyond the medulla, but most of them terminate in tracheoles confined within the limiting sheath. The structure of the medulla suggests that it may represent a greatly modified tracheal ending. The portion of the infundibular space which lies between the infundibular membrane and medullary sheath is bridged by numerous *trabeculae*, which enclose minute canals, the *transinfundibular canals*, uniting the subcellular space with the medullary space. The trabeculae probably do not appreciably retard the flow of fluid in the infundibular space.

By dissection (p. 224) the structures lying within the subcellular membrane may be extracted without other injury than the rupture of the extreme distal portion of the infundibular membrane and of the cortical tracheae as they enter the cellular layer. The term *calyx* is used to denote this internal core of the papilla (Pl. VII, figs. 9, 10; Text-fig. 22 B), consisting of the radial membrane with the muscles attached to it and its extension, the infundibular membrane, with all structures enclosed within it. The term *cortex* may be used to denote the remaining structures consisting of the cellular layer bounded within and without by the subcellular membrane and the external sheath respectively.

Usually a single large trachea supplies each papilla. Close to the papilla it usually divides into several branches. These branches pass towards the centre of the base of the papilla, but before reaching it each gives off one or more

lateral branches. Each lateral branch subdivides into two or more *cortical branches* which perforate the upper part of the infundibular membrane and, passing between the basal cells of the cellular layer, reach the intercellular sinus and ramify within it. These cortical tracheae cease to exhibit chitinous rings within the sinus. The tracheae, after giving off the lateral branches, continue through the medulla as the *medullary tracheae* for about half its length and then divide abruptly into numerous tracheoles without rings. A few of these tracheoles, the *apical tracheoles*, do not supply the medulla, but pass out at its apex and, traversing the lower part of the infundibular cavity, supply the apical portion of the intercellular sinus.

The upper portion of the medullary sheath stretches over and unites the cortical branches and from their inner ends passes on to and unites the lateral branches, thus forming a transverse septum, closing the base of the medulla (Text-fig. 19). Thence the sheath is extended downwards to enclose the main portion of the medulla lying within the infundibular space. Between the points of perforation of the infundibular membrane by successive cortical tracheae the medullary sheath does not extend to the infundibular membrane but leaves gaps, the *semilunar foramina*, affording entrances from the abdominal cavity into the infundibular space (Text-fig. 21, *d*; Pl. VIII, fig. 2).

The suggested circulation of fluid in the spaces mentioned is considered later (p. 231).

(*b*) *Detailed descriptions of the structures composing the papilla*

(1) *The external sheath.*

A thick chitinous membrane, the *external sheath* of Lowne (1895, p. 418), continuous with the intima of the pouch, covers the rectal surface of the papilla.

At its junction with the intima the external sheath exhibits about ninety oval thickenings or *crenations*, separated by short, longitudinally directed, thinner folds. Consequently this portion of the sheath has the appearance of being divided into quadrilateral plates (Text-fig. 21, *v*). These folds pass distally into minute *longitudinal grooves*, which connect with an irregular, longitudinally arranged, network of grooves extending the whole length of the papilla. In general the grooves run nearly parallel to each other, but frequently grooves run obliquely into others, and at intervals fresh grooves arise (Text-fig. 21, *z*). The pattern exhibited by the grooves differs in different specimens, but possibly varies from time to time in the same fly, since it is probable that the grooves serve to allow the sheath to accommodate itself to changes in volume of the papilla. In some cases they cover the surface, except the areas around the bases of the spines, while in others they are most strongly developed at the borders of hexagonal areas marking the boundaries of underlying cells. They are always few and irregular near the apex of the papilla, where the sheath is thick and yellow.

The grooves are clearly visible in fresh preparations and have been noted by Lowne (1869) and subsequent workers. In sections small spaces may occasionally be seen in relation to the grooves between the intima and the cells, and the possibility of these spaces being minute canals has been pointed out. Berlese (1909), Martiis (1924) and Borri (1925), however, regard the spaces as artefacts due to the action of the fixatives. Besides the grooves the external sheath exhibits on its surface a large number, 300 according to Lowne, of groups

of *spines* (Text-figs. 20, 24) which tend to be arranged spirally round the papilla (Borri, 1925). Each group consists of two to eight spines, arranged in a line roughly parallel to the base of the papilla, arising from the distal part of an oval, nipple-like thickening or plate which is strongest at its distal extremity. Each group has, therefore, a comb-like appearance. When viewed from the side each spine is seen to be curved downwards in the long axis of the papilla (Text-fig. 24 H). At the proximal end of the papilla the groups are far apart and their spines few and small, but progressively the groups become more closely set and their spines become longer and more numerous. The groups are most closely arranged and their spines are largest, strongest and most numerous around the apex. Lowne (1869) asserted that a fine pore perforated each spine-bearing plate, but without stating what means of demonstration he adopted. Later (1895, p. 418) he stated that the internal extremities of the pores were "only closed by the large excretory cells," and that the pores were "evidently the excretory orifices of the papillae." Hewitt (1914) also mentioned the pores. On the other hand both Martiis (1924) and Borri (1925) were unable to demonstrate the presence of pores. In these investigations no certain evidence of the existence of pores has been obtained, but in serial sections prolongations of the intercellular sinus may sometimes be seen to approach very closely to the bases of the spine-bearing plates.

It has been suggested that the function of the spines is to perforate or break up the peritrophic membrane within the rectum (pp. 217, 232).

In recently emerged flies the external sheath can be removed easily from the underlying cellular layer, but in older specimens its removal is more difficult. If, however, the opened rectum is placed in weak potash solution the sheath together with the intima of the pouch often becomes completely detached from the other structures (see p. 232). Also after maceration in water the sheath can usually be dissected off with ease.

(2) *The cellular layer.*

The cellular layer is composed of a single layer of very large cells measuring, according to Lowne, 40–80μ in length. The external surfaces of these cells are in contact with the external sheath and their internal surfaces seem to be covered by a very thin membrane, the subcellular membrane, which can only be dissected off with difficulty.

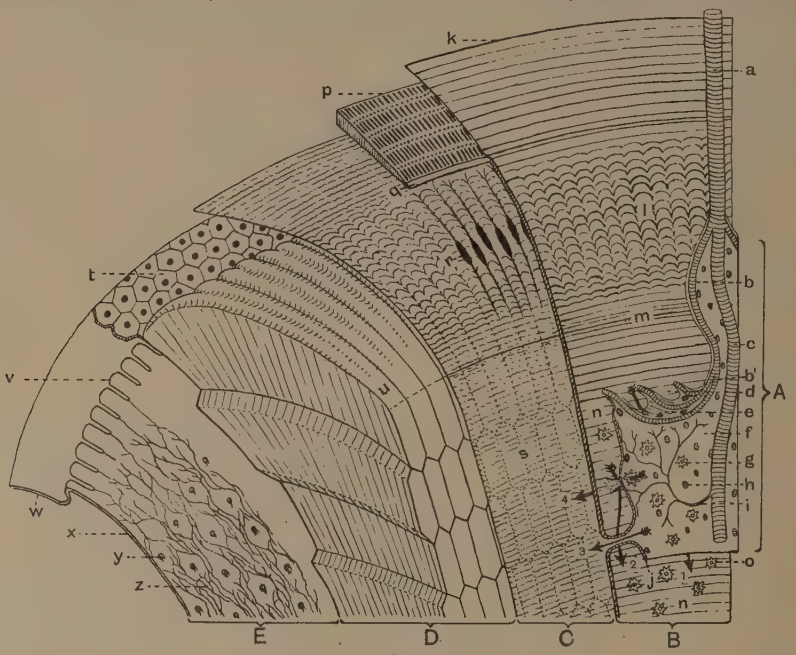
These cells differ in shape in different regions of the papilla.

Basal cells. At the base of the papilla a row of about 45 radially elongated basal cells surrounds the central aperture. Their abdominal surfaces lying beneath the radial membrane are convex in the radial axis and are grooved along the external two-thirds of their length for the accommodation of the radial fibres. Each cell has a central groove and a depression along each margin, which in conjunction with a similar depression along the margin of the adjacent cell forms a groove. Consequently each cell is related to three grooves. Peripherally these grooves seem to be adjusted to the crenations in the external sheath. Centrally the inner surfaces of these cells dip down to become parallel to the external sheath. Their inferior surfaces are shaped so as to accommodate the superior surfaces of the hexagonal central cells (Text-fig. 21, *u*). The grooves in the abdominal surfaces of these cells and their interdigitations with the central cells were illustrated by Martiis (1924).

Central cells. In the central region of the papilla, extending from the basal row to near the apex, each cell has an hexagonal outer surface, slightly elongated in the longitudinal direction, in contact with the external sheath and a similarly shaped internal surface. The margin of the inner surface of each cell is in contact with portions of the margins of six similar cells, and consequently the inner surface of the central part of the cellular layer presents a honey-comb appearance (Text-fig. 21 D and Pl. VIII, fig. 2). The margin of the outer surface of each cell similarly makes contact with six cells, but between the inner and outer margins considerable areas of the intervening lateral surfaces are separated by the intervention of the intercellular sinus (Text-fig. 22, *d*).

Apical cells. Towards the apex the cells progressively lose their hexagonal shape until at the apex they become columnar cells with rounded bases resting on the external sheath. Laterally the apical cells are widely separated from each other, and in fresh preparations they appear to be projecting into the dilated apical part of the intercellular sinus (Text-fig. 20). There are about 700 cells in each papilla.

Each cell possesses a large rounded nucleus, situated nearer the external sheath than the centre of the cell. The nucleus is usually surrounded by a wide clear zone. The cytoplasm in the external part of the cell appears reticulated and often contains granules. That of the internal part frequently shows horizontal striae passing from the inner surface of the cell to the neighbourhood of the nucleus. Borri (1925) has discussed the possible significance of the appearances noted by himself and previous workers in the cytoplasm of these cells.



Text-fig. 21

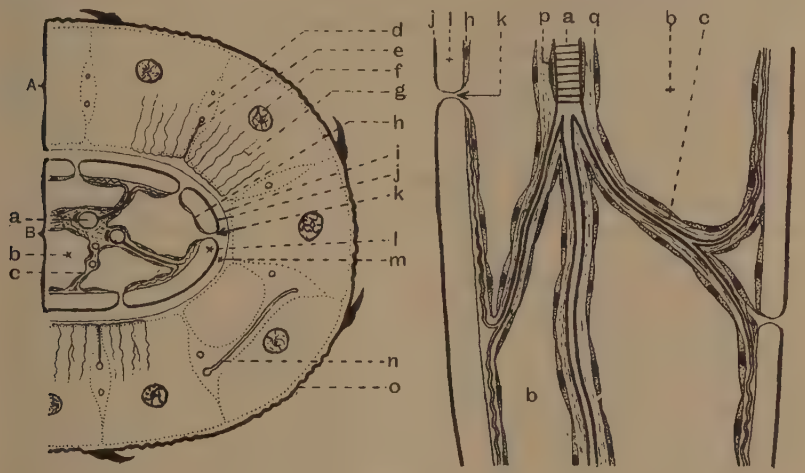
Apart from a few tracheoles supplying the surfaces bounding the intercellular sinus, the cells receive a very definite intracellular supply from small internal lateral branches of the cortical tracheae. These branches after passing internally between the cells divide into numerous tracheoles which spread over and penetrate the inner surfaces of the cells. The tracheoles after penetrating the inner surfaces become very tortuous and pass outwards, some of them reaching as far as the level of the nucleus (Text-fig. 22, *g*). The intracellular tracheoles can be easily traced if the inner surface of the cellular layer, with or without previous treatment with very dilute potash solution, is observed in salt solution under a cover-glass sufficiently supported to prevent pressure on the specimen (Pl. VIII, figs. 7, 8, 9).

More particularly in freshly emerged specimens, it is sometimes possible to strip off portions of the subcellular membrane. This causes separation of the inner margins of the cells, and since their lateral borders are not in contact owing to the interposition of the intercellular sinus the cells can be seen individually projecting from the external sheath

(Pl. VIII, fig. 13). They appear to be arranged in wavy longitudinal lines or cords in accordance with the disposition of the main cortical tracheae (Text-fig. 20). The disposition of the cells and their relation to the cortical tracheae can be well seen in fresh preparations of the papillae of *Fannia canicularis* without dissection.

(3) *The intercellular sinus.*

The intercellular sinus consists of a network of connected spaces lying between the cells of the cellular layer. Commencing between the basal cells about 45 zigzag channels, accommodating longitudinal branches of the cortical tracheae, pass down to the region of the apical cells. These channels are united laterally by transverse communications between the cells through which transverse branches of the tracheae pass (Text-figs. 20, *x'*; 22, *n*). In the apical region the longitudinal channels unite to form a large sac-like dilatation, into which the columnar apical cells project. This sac opens centrally into the lower part of the infundibular space. Thus a passage for fluid exists from the neighbourhood of the basal



Text-fig. 22

cells, by way of the longitudinal channels, to the sac and thence by the infundibular space into the abdominal cavity, or in the reverse direction (Text-fig. 20, arrows).

In descriptions of sections several workers, including Martiis (1924) and Borri (1925), have noted portions of this network of spaces, but have regarded them as artefacts and it is possible that by such methods alone it would be impossible to demonstrate the sinus. In fresh preparations, however, the intercellular sinus can be demonstrated in several ways, and with the knowledge thus gained its distribution, in spite of the great shrinkage[1] which invariably occurs, can be followed to a great extent in serial sections of specimens in which the sinus happens to be especially evident.

The separation of the cells mentioned in the last section after removal of portions of the subcellular membrane indicates the presence of intercellular spaces. In fresh preparations, especially after the removal of the external sheath, the sac-like dilatation of the sinus filled with fluid can be easily distinguished from the cells projecting into it, and the opening

[1] Borri (1925, p. 265) found that a papilla which measured 0·9 mm. in length and 0·5 mm. in diameter at the base was reduced to 0·75 mm. and 0·3 mm. respectively when the xylol stage was reached, and further shrinkage takes place during immersion in paraffin.

of the infundibular space into the sac can usually be observed in *Calliphora erythrocephala*. In smaller flies, such as *Fannia canicularis*, these parts and even the terminal part of the medulla in the infundibular space can be observed readily without removing the external sheath. By puncturing the sac coloured fluid may be made to flow into it and into the infundibular space (p. 229). The most striking demonstration of the ramifications of the sinus is, however, obtained by injecting the infundibular space and sinus from the abdominal cavity (p. 231).

Whether a special lining membrane is interposed between the fluid in the sinus and the cells bounding it has not been determined definitely. The tracheae in the sinus seem to be held in place by delicate strands, and these may perhaps be extensions of a lining membrane, which, if it exists, is not nucleated.

The cells of the cellular layers seem to have a somewhat similar relation to the intercellular sinus as the liver cell cords have to the sinusoids in the mammalian liver.

(4) *The subcellular membrane.*

Associated with the inner surfaces of the cells of the cellular layer there appears to be a delicate membrane, forming the outer wall of a cavity, the subcellular cavity, lying immediately internal to the cellular layer (Text-fig. 22, *i*, *m*). This membrane has not been dissected off as a whole, but, especially in recently emerged or slightly macerated specimens, portions of it may be removed (see p. 222). It seems to extend from the periphery of the basal cells to near the apex of the papilla, where it apparently unites with the apical portion of the infundibular membrane (Text-figs. 20, *r*; 24 C, *6*). There are also indications that this membrane extends beyond the base of the papilla, but whether in relation to the subintimal cells or the muscle sheath has not been decided (Text-fig. 21 C).

(5) *The subcellular space* (Text-figs. 20, *h*, *h'*; 22, *m*).

Whether the inner surface of the cellular layer is covered by a definite membrane or not a definite cavity exists between the cellular layer and the infundibular membrane (Text-fig. 22, *m*). This cavity extends from the margin of the base to near the apex of the papilla, its lower limit being formed by the reflection of the lower part of the infundibular membrane on to the cell surfaces. This cavity, the *subcellular cavity*, does not appear to have been recognised previously. It resembles a vertebrate serous cavity in that little fluid is usually present in it and its walls are consequently in apposition. It is traversed where the basal cells interdigitate with the central cells by the cortical tracheae, and tracheoles from these vessels pass between the cells to branch on its outer wall before passing into the cells (p. 222). No openings apart from the apertures for these tracheae appear to exist on its external wall, which is smooth. Its internal wall, formed by the infundibular membrane, exhibits, however, very numerous openings, the external orifices of the transinfundibular canals. These openings, which are very minute, are surrounded by numerous radiating folds in the membrane, giving them a puckered or star-shaped appearance (Text-figs. 20, *m*, *n*; 24 B). In sections or in dissections it is difficult to detect the lumina of these canals, but their puckered orifices are readily seen. The existence of the canals is best demonstrated by causing coloured fluids to pass through them (p. 230).

Distension of the subcellular cavity with a coagulum has been observed occasionally in sections (Pl. VIII, fig. 12), but the cause of this condition has not been ascertained.

The presence of the subcellular cavity is easily demonstrated by slitting the cellular layer along one side of the papilla down to the calyx, which will be found lying freely for most of its length in a cavity but attached to the cellular layer near the base by the cortical tracheae and at the apex by the thin apical part of the infundibular membrane. By traction on the calyx, after detaching the radial membrane round the periphery of the base, it is often possible to turn the papilla "inside out," thus exposing nearly the whole of the internal

and external linings of the cavity and at the same time demonstrating the reflection of the apical part of the infundibular membrane on to the internal surface of the cellular layer (Text-fig. 24 C).

(6) *The radial membrane and the radial fibres.*

The *radial membrane* is a very distinct sheet of chitin which covers the base of the papilla from its periphery to the rim of its central depression (Text-fig. 20, *b*; Pl. VII, figs. 8, 9, 10; Pl. VIII, fig. 1). Beyond this it is continued as the infundibular membrane (Text-fig. 21, *l*, *m*, *n*). At the outer margin of the base the muscle layers are inserted into a thickening in the membrane. Peripherally the membrane seems to be continued as a very thin sheet over the intra-abdominal surface of the musculature (Text-fig. 21, *k*). Immediately within the muscular insertion the membrane is smooth, but soon it is thrown into a series of radially disposed folds with intervening depressions, corresponding to the grooves in the abdominal surfaces of the basal cells. The grooves cease before the inner margin is reached. There are no structures passing through it or perforations in it.

The *radial fibres* are structures apparently very closely bound to the membrane and lying between it and the surfaces of the basal cells. The fibres have not been isolated and in preparations accompany all accidental folds in the membrane, but can be differentiated by staining with iron haematoxylin. Under most conditions the fibres are very elongated spindles apparently ending in fine divergent fibrillae, attached peripherally to the muscular insertions and centrally to the membrane in the region of the rim. Each fibre possesses near its centre an elongated nucleus with rounded ends, containing a well-marked nucleolus. Frequently there is a small pale-staining area close to the nucleus. Under some conditions, however, the nucleus is found near the peripheral end of the fibre. It is then oval in shape and broader and the fibre itself is swollen at its peripheral end and almost indistinguishable centrally (Pl. VIII, fig. 11). This change may be due to contraction, for in mammalian smooth muscle "during contraction of the cell the nucleus changes its shape; it becomes somewhat oval and folded on its lateral surface and markedly twisted in a spiral direction" (Maximow, 1930, p. 196). Lowne (1869) noted these fibres, stating that two sets of muscle fibres arose from the crenations in the external sheath, "a set from the muscular coat of the rectum and a layer of converging fibres which cover the whole base of the papilla to within a very short distance of its centre and which apparently end in the edge of the membrane forming the boundary of the central cavity, although from the extreme transparency of this membrane it is nearly impossible to be certain of their insertion." Later (1895, p. 417) he says: "The base of the papilla is covered by a layer of radiating muscle fibres, which connect the inner and outer sheaths." He notes that in the exserted ovipositor the papillae may be seen and states (p. 421) that "during life the rectal papillae pulsate rhythmically....The pulsations are due to the contractions of the radiating muscles and the elasticity of the outer capsule; they evidently serve to insure the influx and efflux of blood to and from the central cavity." Owing to the contractions of the pouch musculature it is difficult to be certain of independent movements due to the radial fibres, and such movements have not been recorded by other observers. Martiis (1924) and Borri (1925) considered the radial membrane to be a specialised portion of the peritoneal membrane and recognised the folds in it. Martiis (1924) regarded the nuclei as belonging to the membrane.

(7) *The infundibular membrane and cavity* (Text-figs. 20, *k*, *o*; 22, *j*, *l*).

The *infundibular membrane* is a continuation of the radial membrane, which passes in the form of a funnel to within a short distance of the apex of the papilla, where it is reflected outwards on to the inner surface of the cellular layer to become continuous with the subcellular membrane. As previously stated (p. 224) it forms the inner wall of the subcellular space and is pierced by the cortical tracheae and the transinfundibular canals.

It encloses the funnel-shaped infundibular cavity, forming its outer wall. From the upper two-thirds of its inner surface numerous short hollow *trabeculae*, forming the walls of the transinfundibular canals, pass radially inwards to the medullary membrane. The lower trabeculae are longer and pass more obliquely towards the medulla. These structures, besides transmitting the transinfundibular canals, hold the medulla in position and assure the retention of a uniform channel around it.

In its upper two-thirds the *infundibular cavity* is large, but, except for a narrow marginal channel, is completely occupied by the medulla. In its lower third the cavity is reduced to a narrow tube, within which the "apical tracheoles" of the medulla run. At the basal end of the papilla the cavity opens through the semilunar foramina (p. 227) into the abdominal cavity and distally its lumen is continuous with that of the sac-like dilatation of the intercellular sinus. Experimental evidence of the continuity of these cavities is given elsewhere (p. 231).

The trabeculae were first depicted by Chun (1876). Martiis (1924) and Borri (1925) both illustrated the trabeculae and the membranes between which they lie, but these workers thought that the appearances they observed in sections were due to shrinkage and failed to recognise the nature of the infundibular cavity.

In some of the Syrphidae large trabeculae are present and lateral branches of the medullary tracheae pass through them to reach the intercellular sinus. This suggests that the trabeculae and the transinfundibular canals are structures which originally transmitted lateral branches of the medullary tracheae, but have come to subserve other functions[1].

(8) *The medulla.*

The conical mass of tissue lying mainly within the upper two-thirds of the infundibular cavity has been called the *medulla* (Text-fig. 20). It is divisible into three parts, basal, central and apical. The basal part occupies the central region of the base of the papillae, the other parts lie within the infundibular cavity. The medulla is bounded by the *medullary sheath* which becomes recognisable as a distinct structure at the division of the entering tracheae. It stretches between and unites the tracheal branches entering the medulla as far laterally as the points where the cortical tracheae perforate the infundibular membrane,

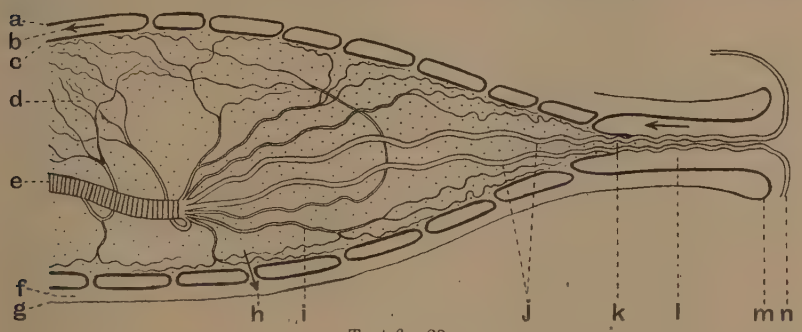
[1] A few observations on the structure of the papilla in the Syrphidae suggest that a detailed study would be of interest.

In form the papilla usually resembles that of *Calliphora erythrocephala*, but in some species it is flattened. The radial membrane is present, but often exhibits neither nuclei, nor radial fibres nor folds. In some species, however, separated, radially disposed, spray-like bundles of fibres belonging to the muscles of the pouch project for some distance on to the membrane and probably perform the same function as the radial fibres. The cortical tracheae, which in general are poorly developed, supply only the basal cells and are not continued as longitudinal trunks in the intercellular sinus. On the other hand the medullary tracheae are very large and as they pass down the medulla give off numerous lateral branches supplied with rings. Each medullary trachea terminates in one or two apical tracheoles. The infundibular membrane and space and the subcellular space are well developed. In some species the trabeculae are large and cone-shaped with their apices reaching the infundibular membrane, and give the medulla a mamillated appearance. The lateral branches of the medullary tracheae traverse many, if not all, the trabeculae and at their apices lose their rings and pierce the infundibular membrane. After crossing the subcellular space these branches pass between cells into the intercellular sinus. In some species they divide in the sinus, and secondary branches, corresponding to the longitudinal trunks of the cortical tracheae in *Calliphora erythrocephala*, run longitudinally for short distances before giving off tracheoles to the inner and outer surfaces of the cells. Some of these terminate as intracellular tracheoles.

In the Syrphidae examined no rectal valve was found, nor were spines seen on the external sheath of the papilla.

thus forming an imperforate sheath over the base of the medulla. Between the points mentioned the medullary sheath does not reach the infundibular membrane, but is reflected inwards, leaving gaps, the *semilunar foramina*, through which the infundibular space is continuous with the abdominal cavity (Text-fig. 19, *h*; Pl. VIII, fig. 2). From this basal portion the sheath is continued downwards in the form of a long bag, slightly constricted near its commencement and pointed at its distal or apical end (Text-figs. 20, 23). It terminates at the entrance to the constricted portion of the infundibular space. The central and apical portions are pierced by the inner orifices of the transinfundibular canals, which are directed obliquely near the apex. At the apex four to eight apical tracheoles apparently pierce the sheath, which, however, may be continued as a delicate membrane over them, and travel down the lower part of the infundibular space into the intercellular sinus, where they separate, and pass up its lateral diverticula (Text-fig. 20, *q*).

The inner surface of the medullary sheath forming the wall of the *medullary space* is lined by flattened cells with oval nuclei (Text-figs. 20, *z*; 22, *h*). The disposition of these cells is best seen by slitting open the medullary sheath and spreading it out before staining. The internal orifices of the transinfundibular canals seem to be lined by thin extensions of these cells. Usually five to seven large *medullary tracheae* with well-marked chitinous rings



Text-fig. 23

pass down to different levels in the central region of the medulla (Pl. VII, fig. 6), where they abruptly break up into numerous branches without rings (Text-fig. 23). About six of these branches pass to the apex of the medulla as the *apical tracheoles*, but most of them, the *medullary tracheoles*, pass outwards towards the inner surface of the medullary sheath and divide into several tracheoles. The latter may divide once or twice but do not branch frequently. Most of these tracheoles, which often form loops, run for long distances and end in very fine twigs, whose terminations have not been traced. They do not seem to anastomose with each other, nor do any of them seem to enter the trabeculae. Lowne (1869), Leydig (1857), Chun (1876), Giacomini (1900) and Borri (1925) claim, however, that they have observed anastomosis.

Delicate chitinous investments appear to surround the medullary tracheae, external to their epithelial layers, the nuclei of which are clearly visible (Pl. VII, fig. 6). Later these investments branch and accompany the larger medullary tracheoles as they pass to the medullary wall. Cells similar to those lining the inner wall of the medullary sheath appear to cover the outer surfaces of these chitinous investments. The medullary space appears, therefore, to be, apart from the openings of the transinfundibular canals, a closed cavity lined by flattened cells, and the tracheae which traverse it seem to be related to it in much the same way as the mesenteric vessels are related to the peritoneal cavity in mammals (Text-fig. 22).

A large nerve, noted by Chun (1876), Leydig (1857) and others, passes towards the medulla together with the tracheae. Lowne (1895, p. 420) states that it is a branch of a nerve which arises from the thoracic ganglion, and, passing through the abdomen, supplies the generative organs and muscle coat of the rectum. He states that he traced branches to the region of the radial membrane, but was unable to trace the nerve into the papilla. In this investigation no attempt was made to ascertain its distribution.

(c) *Experiments designed to aid in the elucidation of the structure of the papilla*

A large number of experiments were carried out from time to time as fresh views required to be tested and previous experiments modified. These experiments are here discussed under various headings, but no attempt has been made to enumerate or describe in detail all that have been attempted.

(1) *Feeding experiments.*

Numerous flies were fed on syrup containing dyes in solution or on bananas soaked in solutions of dyes. The following dyes were used: Janus green (0·25 per cent.), chlorazol sky blue (1 per cent.), trypan blue (1 per cent.), neutral red (1 per cent.), cresol blue (1 per cent.), methylene blue and saffranine. Some of the flies were fed on Indian ink, carmine or graphite in suspension. Some flies were allowed to feed once only, but others were allowed to feed at will for two or three days. Examinations were made at various times after feeding. Frequently the rectal pouch was found distended with brightly stained material or suspension. In some instances certain regions of the gut displayed very different colorations to others, suggesting that useful information on the processes occurring within the alimentary canal might be obtained by suitable experiments with dyes.

The external sheath of the papilla was never found to be stained and no distinct evidence of penetration of a dye into the papilla was ever obtained. It seems, therefore, that the dyes and suspensions used are incapable of passing into the papilla from the rectal cavity.

(2) *Injection of living flies.*

With very fine hollow needles solutions of dyes in salt solution were injected either into the thorax or into the abdomen of living flies. The flies remained alive and not infrequently exhibited considerable activity. They were examined in the following manner after intervals of a few minutes to several hours. After anaesthetisation with chloroform and application of alcohol to the surface, the fly was placed in salt solution and the abdomen opened. The rectum and the hind-gut, which nearly always showed active movements, were removed with the terminal segments and washed thoroughly in salt solution to remove the colouring matter adhering to their intra-abdominal surfaces. The pouch was then opened and the papillae examined and dissected.

Chlorazol sky blue. In the case of flies injected intra-abdominally with this dye (0·5 per cent.) in salt solution and examined after intervals of 30 min. to 4 hours the medulla can be seen as a bright blue cone through the unstained cortex of the papilla. On dissection the dye is found to be present only in the medulla and in the trabeculae. There is no definite evidence of its presence in the infundibular space, cellular layer or intercellular sinus. The dye does not escape from the calyx when it is dissected out and placed in salt solution. In flies injected intrathoracically the same disposition of the dye is encountered. In both cases the musculature of the rectum is stained. It is evident, therefore, that the dye passes by some means into the medullary cavity and thence into the transinfundibular canals but its further progress, if any, has not been traced.

In flies injected intra-abdominally with a strong solution of the dye in gelatin (6 per cent.) and examined at intervals up to 5 hours, only faint staining of the basal portion of the medulla is found. It seems, therefore, that under these conditions the dye is unable to penetrate into the medulla.

Trypan blue. Intra-abdominal and intrathoracic injection of trypan blue (1 per cent.) gave results similar to those obtained with sky blue.

Picro-carmine. Intrathoracic injections cause the medulla and trabeculae to become deeply stained, but the stain does not pass into any other part of the papilla.

Neutral red. In flies injected intra-abdominally or intrathoracically with neutral red (1 per cent.) in salt solution no definite evidence of the presence of the dye in any part of the papilla was found.

Methylene blue. Although the gut and other organs become deeply stained where either intra-abdominal or intrathoracic injections of methylene blue are given the tissues of the papillae remain unstained.

Saffranine. Intrathoracic injections in gelatin (8 per cent.) cause most of the organs to become stained, but no staining of the papilla occurs.

Indian ink. Suspensions of Indian ink injected into the thorax passed into the abdominal cavity, but in dissections no particles were seen in the medulla, and only very rarely were a few particles detected in the upper part of the infundibular cavity.

Carmine. When very thick suspensions in salt solution are injected into the abdominal cavity particles are never found in the medulla, but in some cases, especially if the fly has been kept alive for 30 min. or longer, a few particles may be found in the infundibular cavity. Some of these particles exhibit Brownian movements and some move slowly along the cavity avoiding the trabeculae. Occasionally they penetrate as far as the contracted portion of the cavity, but they have not been observed to enter the sinus. It is remarkable how little of the very thick suspension filling the abdominal cavity enters the infundibular cavity in spite of the increased intra-abdominal pressure caused by the injection. Particles were found in the positions mentioned in a fly injected with a thick suspension in gelatin (6 per cent.) and kept at 37 per cent. for 90 min.

The experiments suggest (1) that while fluids containing some dyes in solution can pass from the abdominal cavity into the medulla the mechanism is such that other dyes in solution as well as gelatin and particles in suspension are excluded, and (2) that by the method employed very little of the inoculum is forced through the semilunar foramina into the infundibular space. The progress of those dyes which enter the medulla has not been traced beyond the transinfundibular canals either because changes in colour occur or because the quantity passing into the subcellular space at any time is very small.

(3) *Experiments with freshly dissected tissues.*

In order to throw further light on the channels within the papilla experiments were made on tissues removed from anaesthetised flies.

(i) *To demonstrate the continuity of the apical dilatation of the intercellular sinus with the infundibular space.* In a few instances the rectal pouch was opened and the external surface coated with vaseline in order to prevent the entry of stain through the basal region. The apex of the papilla was then brought into contact with a drop of sky blue or methylene blue solution and its tip punctured with a fine Hagedorn needle. In successful experiments the stain immediately penetrated into the sac-like dilatation of the sinus and passed up the infundibular space, thus demonstrating their continuity. The stain only passed to a very limited extent into the lateral diverticula of the sinus between the cells and never entered the transinfundibular canals unless the subcellular cavity had been opened accidentally.

(ii) *To confirm the results of inoculation experiments.* In all the following experiments, which were very numerous, the abdomen of an anaesthetised fly was opened under salt solution and the terminal portion of the gut from the origin of the Malpighian tubes to the

anus, still attached to the terminal segments, removed with as little injury as possible. These preparations, in which active contractions of the pouch musculature usually continued for long periods, were placed in saline or gelatin solutions of the dyes or in the suspensions to be investigated. After removal from the solution or suspension the preparations were thoroughly washed in salt solution to remove any coloured fluid clinging to their external surfaces and dissected. Sometimes the preparations were kept at 37° C., but this did not influence the results. The external sheath never showed any trace of staining.

Sky blue. In almost all the very numerous experiments with saline solutions (0·5 per cent.) of this dye the medulla and trabeculae became bright blue, but the other parts of the papilla remained unstained. Even the nuclei within the medulla either remained unstained or became only very lightly stained. If, however, the preparations, after washing, were left in salt solution the cells of the cellular layer and their nuclei assumed a greenish-blue colour. Also, if the preparations were allowed to remain in the stain for some hours all the tissues were stained blue.

Though very active movements often persisted for some hours in preparations immersed in sky-blue solutions in gelatin (6 per cent.) little stain penetrated into the medulla, unless immersion was prolonged for 2 hours or more.

Janus green. After an immersion of 1 hour in a saline solution (0·5 per cent.) of this dye no staining of the medulla or of the cellular layer was observed, though the stain had been taken up by the pouch muscles and the radial fibres.

Neutral red. After immersions in saline solutions (1 per cent.) for 15 min. no staining of the medulla was observed, but after immersions exceeding this time slight coloration of the basal cells of the cellular layer became evident, apparently indicating that the stain had commenced to penetrate the radial membrane. Progressive coloration of the cellular layer continued until, after 3–5 hours' immersion, all the cells were pink, but even after these periods only the most superficial portion of the basal part of the medulla was deeply stained. Preparations immersed in gelatin solutions gave very similar results.

Methylene blue and eosine. These dyes behaved very similarly to neutral red.

Indian ink. After immersions in thick suspensions for periods ranging up to 120 hours no particles were found in the medulla. Occasionally very small numbers of particles were found in the upper part of the infundibular space.

Carmine. In very thick suspensions of carmine in gelatin (6 per cent.) very active contractions of the muscles continued for some hours, but particles were never found in the medulla and very few were observed in the infundibular space.

These experiments, like the inoculation experiments, show that while some dyes very readily pass into the medulla others penetrate with difficulty and also that only occasional particles from thick suspensions pass into the infundibular space.

(iii) *To observe the passage of fluids and particles in suspension through the transinfundibular canals.* Many experiments of the following type were carried out.

The subcellular cavity is opened and the calyx, either attached to the cellular layer by the terminal part of the infundibular membrane or with that attachment severed, is placed on a slide in a small drop of salt solution. Most of the fluid surrounding the preparation is then removed with filter-paper, and from an adjacent drop of dilute stain a very small quantity is led with the point of a needle till it comes into contact with a small area on the side of the calyx. The trabeculae immediately become coloured and very shortly afterwards stain appears within the medulla and slowly spreads from the area first affected. By removing as far as possible the stain in contact with the calyx with filter-paper and bringing a small drop of a differently coloured stain into contact with the same area the trabeculae and the adjacent parts of the medulla may be made to take a corresponding colour. On the other hand the replacement of the first stain by salt solution causes the trabeculae to become almost colourless, while the colour is retained within the medulla though the area near the affected trabeculae becomes paler.

When a calyx into which very dilute stain has been passed in the manner described above is allowed to dry slowly on a slide the trabeculae remain refractile as if containing fluid for hours after the infundibular membrane has lost its normal appearance. No air has, however, been seen to enter them, presumably because the minute orifices of the canals become sealed when the infundibular membrane dries.

The passage of coloured fluid through the trabeculae into the medulla may be observed in preparations mounted in salt solution under supported cover-glasses when stain is caused to run in from one side.

In *Sarcophaga* the trabeculae are few, large and conspicuous and the passage of stains can be more easily studied than in *Calliphora*.

Few experiments with suspensions were made because the particles adhere so tightly to the puckered orifices of the canals that washing fails to remove them. It was therefore difficult to ascertain whether penetration into the canals had occurred or not.

Experiments of this type show that fluid can pass easily through the transinfundibular canals and that these canals are in communication with the medullary space. All stains behaved in the manner described. Under these conditions stain never enters infundibular space.

(iv) *To demonstrate the relation of the infundibular space to the distal portions of the intercellular sinus.* Though experiments had demonstrated that under certain conditions small particles could pass from the abdominal cavity into the infundibular space, the communication between the infundibular space and the portions of the intercellular sinus lying between the cells had not been established experimentally. This was successfully demonstrated in the following manner.

A very fine suspension of graphite, prepared by Drinker and Churchill's (1927) method, was placed in a small wide-mouthed bottle, and either flies with the abdomen opened or preparations of the rectal region, such as those described in section (ii), dropped in. The bottle was then placed in a strong metal box connected by taps to the outside air, an exhaust pump and a carbon dioxide cylinder. After the pressure within the box had been alternately and rapidly decreased and increased to 70 lb. on several occasions the preparations were thoroughly washed in salt solution and examined. Often some of the suspension was found to have penetrated into the lower part of the infundibular cavity and in these cases by slight compression of the base of the papilla the suspension could be made to pass into the dilated portion of the sinus occupying the apex of the papilla. When the pressure on the base was released the suspension returned into the infundibular cavity. Occasionally, however, the suspension was found to have filled the dilated portion of the sinus, so that the cells projecting into it appeared as clear areas on a dark background. In such preparations slight pressure on the base of the papilla caused the suspension to pass up towards the region of the basal cells, following the courses of the main branches of the cortical tracheae. On releasing the pressure most of the suspension returned to its original position, but a little remained outlining the ramifications of the sinus (Pl. VIII, figs. 4, 5).

Such experiments prove that a free passage exists from the upper part of the intercellular sinus surrounding the basal cells through its expanded sac-like apical portion and the infundibular space into the abdominal cavity.

(*d*) *Circulation of body fluid within the papilla*

The structure of the papilla and the experiments which have been quoted suggest that there is a circulation within the organ, fluid from the abdominal cavity, containing selected constituents, entering the medulla and passing by way of the transinfundibular canals into the subcellular cavity from which it presumably reaches the intercellular sinus by traversing the cells of the

cellular layer. From the intercellular sinus it escapes into the infundibular space and again enters the abdominal cavity by way of the semilunar foramina. If such a circulation exists it suggests that the very large cells of the cellular layer function in some respects like liver cells, since they are related to the intercellular sinus in much the same way as the cords of liver cells are related to the sinusoids.

(*e*) *Maceration*

When the rectal pouch or a rectal papilla with part of the pouch wall attached is placed in dilute potash solution the external sheath gradually becomes separated from the other tissues of the papilla. The separation commences at the apex, fluid apparently accumulating between the sheath and the apical cells. The latter seem to be forced towards the base, especially centrally, where a basally directed pocket is formed. The changes observed in the early stages suggest that increasing pressure within the sheath gradually drives fluid out of the intercellular sinus until its sac-like apical enlargement is obliterated and the cells covering it are forced into the pocket thus created. At the same time compensatory bulging of the central region of the base occurs (Text-fig. 24 E). As the separation of the cells from the sheath progresses the bulging of the base increases (Text-fig. 24 F), until finally the external sheath, together with the intima in its neighbourhood, becomes completely separated from the other tissues composing the papilla, to which the pouch muscles remain adherent. The cells to a great extent regain their normal disposition (Text-fig. 24 G). The same sequence of events occasionally occurs after prolonged maceration in salt solution or water. Whether the fluid enters the sheath by the pores described by Lowne has not been ascertained.

This action of potash solution has been noted and the final stage illustrated by Borri (1925).

(*f*) *The peritrophic membrane in the rectum*

The peritrophic membrane passes, apparently uninjured by the spines of the valve region (p. 214), through the first part of the rectum into the pouch. In many cases it is difficult to trace within the pouch. Occasionally, however, it may be found, apparently intact, forming a double loop, first passing to the distal extremity of the pouch and then forwards to the proximal extremity and finally again passing backwards to enter the anal part of the rectum. Even in these cases the presence of masses of bacteria on the surfaces of the papillae or of particles of food, such as Indian ink, in temporary pockets round their bases suggests that these materials have escaped from the membrane through perforations or considerable ruptures, presumably caused, as suggested by Engel (1924), by the comb-like spines of the external sheaths of the papillae.

(*g*) *The function of the rectal papillae*

Many different suggestions as to the function of the rectal papillae or glands have been made, and Wigglesworth (1932) has reviewed the literature on the subject and has adopted the view that their function is to reabsorb water from the rectum. In this section only a very brief review is, therefore, given.

In *Calliphora erythrocephala* and in most Diptera the rectal papillae are very conspicuous objects, but in many insects they are inconspicuous or apparently absent. "It is frequently stated (Tonkov, 1923; Borri, 1925) that the irregular occurrence of the rectal glands adds to the difficulty of determining their function. Thus, they are said to be absent from nearly all larvae,

most Coleoptera, and all Hemiptera. But, as was pointed out by Chun (1876), wherever discrete rectal glands are absent, the entire rectum is lined by a uniform columnar or cubical epithelium of the same type as that composing the rectal glands; whereas, when the rectal glands are present, the intervening epithelium is more or less vestigial. It is therefore reasonable to suppose that this uniform epithelium and the rectal glands subserve a common function" (Wigglesworth, 1932).

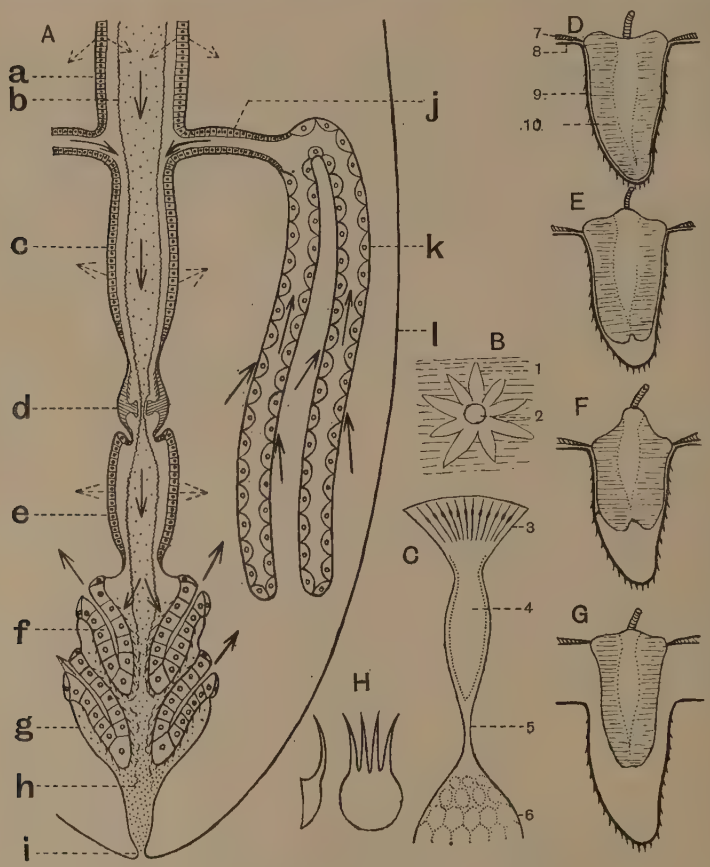
Chun (1876), Lowne (1895; uric acid), Petrunkewitsch (1899; a secretion aiding defaecation), Trappmann (1923) and Abbott (1926), working with various insects, suggested that the papillae had secretory functions. They, however, produced little evidence in support of their views, and "all observers have agreed that no digestive enzymes are produced in the rectum and that the excretory products are derived from the Malpighian tubes" (Wigglesworth, 1932, p. 145). Leydig (1857) suggested that they might be respiratory, and Martiis (1924) that they might be endocrine organs. Berlese (1909) thought the papillae might grip the peritrophic membrane and aid in drawing it down, and Engel (1924) considered that the spines of the external sheath might tear the membrane and thus aid in its expulsion from the anus.

Berlese (1909) put forward the view that the papillae absorb products of digestion from the rectum, and Borri (1925), thinking that the great differentiation of the cellular layer and the very large tracheal supply implied important functions, made extracts of the papillae of numbers of blow-flies fed on meat and tested them for lipoids, and also tested by staining methods the papillae of flies fed on sugar, fat meat and milk for glycogen and fat; all with negative results. He therefore came to the conclusion that these organs were not concerned with the passage of such substances from the rectum.

While others had demonstrated that absorption of water from the rectum took place in certain larvae (Frenzel, 1882; Verson, 1905; Metalnikov, 1908) Wigglesworth (1931) showed that a circulation of water occurred in the blood-sucking bug *Rhodnius prolixus*, and put forward the view that "reabsorption of water might prove to be the function of the so-called 'rectal glands,' which would thus play an essential part in the all-important process of water conservation in insects" (1932, p. 131). In support of this view he has collected evidence bearing on the reabsorption of water from the rectum in members of several orders of insects, Thysanura, Dermaptera, Orthoptera, Neuroptera, Coleoptera, Lepidoptera, Diptera, Hymenoptera, Siphonaptera and Anopleura. In regard to Cyclorrhaphous Diptera he noticed that in the late stages of pupal development of *Lucilia sericata* the hind-gut and rectum become greatly distended with fluid, which later contains spheres of uric acid. Shortly before emergence, however, the "rectum is filled with a semi-solid mass of uric acid in a small quantity of deep yellow fluid." He says that "it is difficult to account for these observations without supposing a continuous reabsorption of water from the rectum; and such absorption must be ascribed to the rectal glands" (p. 139). In regard to the adults after emergence he says that "these

flies lose fluid rapidly and do not survive for more than a day or two unless given plenty of food and water." He also noted that those flies which evacuated the fluid from the rectum died the quickest, and argues that they died because "this fluid" was "no longer available to the insect."

He further points out that "there is no difficulty in accepting the passage of water through the chitinous intima of the hind-gut; for it has been shown



Text-fig. 24

repeatedly (Gorka, 1914; Abbott, 1926; Eidmann, 1922) that the thin cuticle of the insect hind-gut is freely permeable to water."

Wigglesworth's view is illustrated in Text-fig. 24 A, which reproduces his Text-fig. 1, in a modified form in order to represent the conditions existing in *Calliphora erythrocephala.*

Wigglesworth, who has "tacitly accepted" Chun's opinion that "the uniform epithelium," which may replace the papilla, "and the rectal glands

subserve a common function," has brought forward a most interesting hypothesis, but, at least in the case of the adult Cyclorrhaphous Diptera, he has produced no evidence that the papillae are the organs concerned in the reabsorption of water. In these insects the first part of the rectum resembles the hind-gut in structure and reabsorption probably occurs there, and possibly it also occurs through the epithelium lining the pouch apart from the papillae. These insects are provided with large crops from which fluid can be taken into the gut as required and consequently in them the conservation of water is probably not so essential as in many other insects. Even if it is shown that in these insects reabsorption of water is one of the functions of the papillae their complicated structure seems to indicate that they have other important functions.

C. THE THIRD OR ANAL PART OF THE RECTUM

The third part of the rectum is a narrow tube of nearly uniform diameter, lined by an intima similar to that in the pouch, which is normally thrown into very numerous folds, and provided with strong internal longitudinal and external circular muscle coats, continuous with those of the pouch. This part is about the same length as the pouch and terminates at the anus, where the intima becomes continuous with the integument. Near the anus the muscle coats are slightly thickened to form a sphincter.

XII. ARRANGEMENT OF THE GUT IN DIFFERENT SPECIES

In some species the central portion of the mid-gut within the abdomen is coiled into a tight helix, while in others the helicoid portion is less developed or may be represented by an acute flexure (Text-fig. 10). Transposition of the abdominal viscera was noted in one specimen of *Calliphora erythrocephala*.

The length of the hind-gut in relation to the length of the mid-gut apparently varies greatly, depending on the position of the rectal valve, which may be situated within a short distance of the pouch or may be advanced so far as to lie midway between the pouch and the Malpighian tubes. If the portion of the gut lying between the pouch and the rectal valve, here described as the first part of the rectum (p. 204), is regarded as functionally a part of the hind-gut the relation is not very dissimilar in most of the flies observed.

The lengths in mm. of the various regions of the alimentary canal in large specimens of four species are given below:

	Calliphora erythrocephala	*Musca domestica*	*Fannia canicularis*	*Sarcophaga carnaria*
Oesophagus	3·0	1·75	1·5	3·5
Mid-gut from proventriculus	28·0	19·0	10·0	27·0
Hind-gut	4·5	1·25	1·0	1·5
Valve region	1·5	0·75	0·5	1·5
First part of rectum	1·0	1·0	1·0	2·5
Rectal pouch	1·5	0·75	0·75	1·25
Anal part of rectum	2·0	1·0	0·5	1·75

XIII. THE AORTA

Though Lowne (1895, p. 638) stated that the aorta was "a muscular tube," no detailed account of its musculature seems to have been published. In the case of *Calliphora erythrocephala* the vessel can be dissected out, split from its junction with the "heart" for nearly the

whole length of its thoracic course, and stained in the manner described. In such preparations the musculature has the appearance of being arranged in "sections." Along each section run two to four narrow, longitudinal, striated bands, which at each end break up into lateral and oblique branches. Some of the oblique branches re-unite to form the longitudinal bands of the next section. Throughout each section there are numerous very fine, transverse, striated bands, many of which undoubtedly arise as lateral branches either of the main longitudinal bands or of short, thin, longitudinal bands which are not uncommon near the ends of the sections. A single large, oval nucleus, or occasionally two nuclei placed close together on opposite sides of the tube, is present near the centre of each section (Pl. VI, fig. 4). "In view of the fact that the thoracic vessel is itself to be considered as a modified anterior portion of the heart" (Minchin, 1905) it is possible that each section represents an area bounded by a large cell "with striated muscle fibrillae differentiated within the protoplasm," though the longitudinal fibrillae are continuous with those of adjoining sections.

XIV. SUMMARY

A detailed description of the musculature in the different regions of the alimentary canal of *Calliphora erythrocephala* is given, and an account of the structure and function of the crop, proventriculus, ducts of the Malpighian tubes, rectal valve and rectal papillae. It has been shown by dissections and experiments that a system of channels exists in the rectal papillae through which the body fluid probably circulates, and it is suggested that the very large cells may have functions resembling those of liver cells.

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EXPLANATION OF TEXT-FIGURES 1–24

Text-fig. 1. Diagram illustrating the alimentary canal of *Calliphora erythrocephala* as seen after removing the dorsal integument. The head is turned to the right with the proboscis extended. In the thorax the proventriculus and thoracic ventriculus, which lie dorsal to the crop-duct, have been displaced to the right, and in the abdomen the helicoid region has been slightly displaced to the right. The diameter of the gut has been somewhat reduced, and no attempt has been made to reproduce the intricate arrangement of the Malpighian tubes.

1. Inner side of the labellum with pseudotracheae. *2*. Labial salivary gland. *3*. Cavity of labial gutter. *4*. Pharyngeal cavity. *5*. Dilator muscle of pharynx. *6*. Retractor muscle of oesophagus, arising from the remains of the ptilinal sac. *7*. Pre-ganglionic portion of the oesophagus. *8*. "Brain." *9*. Ganglionic portion of the oesophagus. *10*. Post-ganglionic portion of the oesophagus. *11*. Lingual salivary gland. *12*. Crop-duct. *13*. Crop. *14*. Hind-gut. *15*. Malpighian tube. *16*. Lingual salivary gland (abdominal portion). *17*. Rectal valve. *18*. First part of rectum. *19*. Rectal pouch, containing the rectal papillae. *20*. Anal portion of rectum. *21*. Salivary pump with afferent and efferent salivary ducts and gracilis muscle. *22*. Proventricular portion of oesophagus. *23*. Proventriculus. *24*. Adductor muscle of thoracic ventriculus. *25*. Thoracic ventriculus. *26*. Helicoid region of mid-gut. *27*. Post-helicoid region of mid-gut. *28*. Abdominal ventriculus. *29*. Ventricular loop, ascending limb.

Thickenings in the oesophageal and crop-duct walls mark the positions of the anterior and posterior oesophageal, proventricular and crop-duct sphincters.

Text-fig. 2. Side view of the junction of the post-ganglionic portion of the oesophagus (A) with the proventricular portion (B) and the crop-duct (C), showing the musculature. Near its junction with the proventricular portion the flat, circular muscle bands (*1*) of the post-ganglionic portion become thicker and coarser to form the *posterior oesophageal sphincter* (*2*). Below the junction the fibres are irregularly disposed (*3*) and thin, longitudinal bands (*4*) arise and pass up the proventricular portion. The circular bands near the origins of the proventricular portion and the crop-duct are coarse and thick, forming the *proventricular* (*5*) and *crop-duct* (*6*) *sphincters* respectively. In the crop-duct the flat bands (*7*) are so arranged as to give an interlacing appearance. Occasionally short longitudinal bands (*8*) are formed.

Text-fig. 3. Musculature of the crop-duct in the posterior part of the thorax. The arrow indicates the long axis of the gut.

Text-fig. 4. Crop fully contracted.

Text-fig. 5. Diagram illustrating the distribution of the muscle bands over the surface of the distended crop. On the left is shown the dorsal surface, which becomes anterior when the crop is distended (see Text-fig. 10 A). *1*. Narrow parallel bands. *2*. Intervening network. *3*. Broad bands. *4*. Longitudinal bands covering the end of the crop-duct. On the right is shown the ventral surface covered by narrow parallel bands. Some of the bands covering the upper pole (*5*) become longitudinal and are inserted into the upper transverse bands.

Text-fig. 6. Illustrating the musculature of the crop.

A. A portion of the network (*2*) connecting narrow, parallel bands on the left (*1*) with broad bands on the right (*3*).

B. Portions of five narrow, parallel bands (*1*) showing the spaces (*4*) between them bridged by numerous communications of their sheaths. In most of these communications (*5*) no muscle fibres are present, but some transmit single fibres (*6*), others small bands (*7*) and others a number of separated fibres (*8*). Occasionally fibres pass to distant parallel bands (*7*). Sometimes when the sheath widens the fibres composing the band are seen to be separated by spaces (*9*).

C. One of the narrow parallel bands (*10*) terminating by breaking up into branches. Some of the branches join distant parallel bands, others seem to end in fine fibrillae attached to adjacent muscle sheaths (*11*). To avoid confusion the central portions of the neighbouring bands are indicated in outline only.

D. Unusually complicated arrangements of fibres passing into lateral communications.
E. A group of the cells underlying the muscle layer.
F. Part of a broad band from a contracted crop (on a smaller scale).

Text-fig. 7. A and B illustrate the proventriculus and anterior portion of the thoracic ventriculus as seen from the left side after removal of the thoracic muscles, air sacs and salivary glands. The organs have been slightly tilted to the right in order to exhibit the hilum of the proventriculus. A illustrates a fresh preparation and B a preparation stained to show the musculature. In the latter the longitudinal muscles are continued along the ventriculus as far as *s*; the transverse muscles are shown as far as the commencement of the ventriculus, but are discontinued between this region and *s*. Beyond *s* they are shown uncomplicated by the presence of the longitudinal muscles. C, as far as *v*, illustrates the under-surface of the structures as seen by transmitted light in fresh preparations. From *v* to *s* the longitudinal and transverse musculature is indicated, and beyond *s* the transverse musculature only. In these figures only the most prominent pouches of the transverse projecting folds in the ventriculus are indicated but the extent of the folds and the relation of pouches to them may be judged by reference to the spaces between the transverse muscles in B.

a. Adductor muscle. *b*. Aorta. *c*. Proventricular ganglion. *d*. Post-ganglionic oesophagus. *e*. Nerve entering the proventriculus. *f*. Nerve passing along the crop-duct to the crop. *g*. Proventricular oesophagus. *h*. Hilum of proventriculus. *i*. Basal ring muscle. *j*. Crop-duct. *k*. Margin of proventriculus. *l*. Trachea passing over the surface of the proventriculus and under the basal ring muscle to enter the hilum. *l'*. Tracheae supplying the thoracic ventriculus. *m*. Small air sac from which these tracheae arise. The hatched circle in the middle of it represents its communication with the great longitudinal thoracic air sac. *n*. Thoracic ventriculus. The broken line points to the margin of one of the pouches on its surface. *o*. Deep longitudinal muscle of proventriculus. *p*. Superficial longitudinal muscle band. *q*. Circular muscle covering the vault of the proventriculus. *r*. One of the longitudinal muscle bands of the ventriculus. *s*. Point beyond which the longitudinal muscles are not illustrated. *t*. Transverse muscle of the thoracic ventriculus. *u*. Decussation of transverse muscles. *v–s*. Region in which (C) both longitudinal and transverse muscles are illustrated. *w*, *x*. Opaque rings noticed in specimens examined by transmitted light and caused by the upper and lower flanges of the plug respectively.

Text-fig. 8. A. Muscles of the outer wall of the proventriculus and of the anterior part of the thoracic ventriculus. B. Proventriculus as seen when the intussusception of the plug has been reduced by traction on the oesophagus. C. Vertical section of B. D. Proventricular oesophagus extracted by traction from the plug. E. Transverse section of the proventricular oesophagus, hilum and lower flange of the plug at the level of C in Text-fig. 9.

1. Longitudinal muscle bands passing on to the thoracic ventriculus. *2*. Transverse muscle bands of the ventriculus separating pouches. *3*. Outline (dotted) of one of the pouches. *4*. Superficial longitudinal muscle bands on the vault of the proventriculus. *5*. Circular muscles of the vault of the proventriculus. *6*. Superficial longitudinal bands on the lower part of the proventriculus. *7*. Deep longitudinal muscle of the proventriculus. *8*. Basal ring muscle. *9*. The portion of the proventricular wall which normally forms the outer wall of the hilum. The dotted lines indicate the apparent fine prolongations of the intrinsic longitudinal muscles of the plug to the basal ring muscle. *10*. Proventricular oesophagus. *11*. One of the tracheae passing over the wall of the proventriculus and under the basal ring muscle to enter the plug. *12*. Upper epithelial pad. *13*. Lower epithelial pad. *14*. Plug. *15*. Upper flange of plug. *16*. Lower flange. *17*. Dorsal foramen of plug; the ragged edge seen at *17'* in D is caused by the intima of the proventricular oesophagus tearing round the margin of the dorsal foramen, beyond which the chitin is firmly adherent to the cells of the plug. *18*. Dilated portion of the proventricular oesophagus with portions of the intrinsic muscles adhering to it. *19*. Section of lower flange. *20*. One of the tracheae traversing the hilum. *21*. Cavity of the hilum. *22*. Circular muscle of the proventricular oesophagus. *23*. A longitudinal fold of the proventricular oesophagus bounded by the intima and containing longitudinal muscle fibres. *24*. Lumen of proventricular oesophagus.

Text-fig. 9. Diagram illustrating a vertical longitudinal section through the proventriculus of *Calliphora erythrocephala*. On the left side of the plug the distribution of the intrinsic muscles is shown and on the right the distribution of the tracheae.

1. Lumen of the thoracic ventriculus. *2*. Peritrophic membrane in section. The upper part is continued to its origin, but, in order to avoid confusion, the lower part is not continued after entering the proventriculus. *3*. Epithelium lining the ventriculus. *4*. One of the transverse muscle bands of the ventriculus. *5*. One of the longitudinal muscle bands of the ventriculus. *6*. Circular muscle of the vault of the proventriculus. *7*. Superficial longitudinal muscle of the proventriculus (continuous with *5*). *8*. Deep longitudinal muscle of the proventriculus. *9*. Upper epithelial pad. *10*. Depression between the epithelial pads. *11*. Lower epithelial pad. *12*. Basal ring muscle. *13*. Cavity of hilum. *14*. Crop-duct. *15*. Intima of crop-duct. *16*. Cells underlying intima. *17*. Muscle of crop-duct. *18*. Sphincter of crop-duct. *19*. Posterior sphincter of oesophagus. *20*. Post-ganglionic oesophagus. *21*. Muscle of oesophagus. *22*. Cells underlying intima. *23*. Intima of oesophagus. *24*. Aorta. *25*. Proventricular ganglion. *26*. Union of the layers of the peritrophic membrane originating from the upper and lower epithelial pads. *27*. One of the tracheae supplying the plug. *28*. Neck of the proventriculus, where it unites with the ventriculus. *29*. Upper flange of plug. *30*. Cavity between the flanges, which accommodates the lower epithelial pad. *31*. Lower flange of plug. *32*. Dorsal foramen of plug. *33*. Intrinsic radial muscles of the plug. *34*. Tracheal branches passing into the plug. *35*. Intima of proventricular oesophagus. *36*. Cells underlying the intima. *37*. Longitudinal muscle. *38*. Circular muscle of proventricular oesophagus, thickened to form a sphincter. *39*. Entrance to the proventricular oesophagus. *40*. Network formed by branches of the cells of the plug.

Insets. A, B and C illustrate sections through the plug in the directions indicated by the arrows at the points A, B, C in the large figure. In each case tracheae (*a*) and muscles (*b*) are shown passing through irregular spaces in the network formed by the elongated bases of the cells.

Text-fig. 10. Diagram illustrating the arrangement of the gut within the abdomen in (A) *Calliphora erythrocephala* (side view) and in (B) *Fannia canicularis*, (C) *Musca domestica* and (D) *Sarcophaga carnaria* (dorsal view). The rectal valve is shaded. The last three figures illustrate the reduction of the helicoid turns from the condition found in *Fannia canicularis* to the acute flexure in *Sarcophaga carnaria*.

In figure A the position of the crop when empty is indicated by the continuous line (*1*), and when distended by the dotted line. The shape of the abdomen when the crop is distended is indicated by the broken line. *2*. Descending limb of the ventricular loop.

Text-fig. 11. *Calliphora erythrocephala*. I. Longitudinal section in the helicoid region of the mid-gut, showing elongated, vacuolated cells. *a*. Inner circular muscle bands. *b*. Outer longitudinal muscle bands. II. The junction of the mid- and hind-gut in a fresh specimen. *c*. Mid-gut. *d*. Malpighian duct. *e*. Smooth anterior portion of the hind-gut, corresponding to the transitional musculature. *f*. Hind-gut showing corrugations due to the contracted condition of the muscle layer. III. Transverse section of the hind-gut with the muscle layer in a state of contraction. *g*. Muscle layer which only follows to a slight extent the infoldings of (*h*) the epithelial layer. *i*. Peritrophic membrane.

Text-fig. 12. Diagram illustrating the musculature of the common duct of two Malpighian tubes, as seen when the structures have been slit open and the epithelium removed.

a, *b*. Two Malpighian tubules uniting before opening into the common duct. The dotted outlines indicate the approximate positions of the cells of the tubules. *c*. Distal circular muscle band of the duct, with some of its fibres terminating in fan-shaped expansions. *d*. A fibre passing obliquely between two circular bands. *e*. Space between two circular bands. *f*. Common duct. *g*. A longitudinal muscle band. *h*. Nucleus of a circular muscle band. *i*. A fibre of a circular band terminating in a fan. *j*. Fan-like termination of a fibre enlarged.

Text-fig. 13. Diagram showing the musculature at the junction of the mid-gut (left of *d*) and the hind-gut (right of *d*). The gut has been opened longitudinally and the epithelium and intima removed.

a. A longitudinal muscle band of the mid-gut. *b*. A nucleus in a circular muscle band of the mid-gut. The nuclei are arranged in four lines. *c*. A circular muscle band of the mid-gut. *d*. Distal margin of the last circular muscle band of the mid-gut. On the internal surface of the gut the proximal margin of the intima of the hind-gut reaches this level. *e*. Some fibres of the last circular band together with branches from the neighbouring longitudinal bands forming loops distal to the orifices of the Malpighian ducts. *f*. Transitional region in which fine branches of the longitudinal muscle bands of the mid-gut pass distally to reach the transverse bands of the sphincter. In this region circular bands are not found. *g*. Proximal, closely arranged, transverse muscle bands of the hind-gut, probably functioning as a sphincter. *h*. Normal musculature of the hind-gut, consisting of large transverse bands, some ending in stellate expansions, connected together by smaller longitudinal branches. *i*. A fine strand from one of the muscle sheaths passing down to the basement membrane. *j*. Three communications between adjacent muscle sheaths. These communications are very numerous, but to avoid confusion have been omitted elsewhere. *k*. A trachea passing down between muscle bands to break up into tracheoles on the basement membrane. *l*, *l'*. Orifices of the Malpighian ducts.

Text-fig. 14. Semi-diagrammatic illustration of the musculature of the last part of the hind-gut, rectal valve and first part of the rectum.

a. Distal portion of the hind-gut, showing irregular transverse bands of various sizes with oblique and longitudinal branches. *b*. Transitional region in which the lateral branches become almost wholly longitudinal and finally cease, and the transverse bands become less branched and more closely approximated. *c*. Region of the rectal valve with broad, regular, closely approximated, non-branching transverse bands. *d*. First part of the rectum (commencing at the arrow) with similar but less closely approximated transverse bands. The very numerous communications between their sheaths have been omitted. *e*. Distal end of the first part of the rectum, showing the origin by numerous deep lateral branches of the inner, longitudinal muscles of the pouch (*f*). In this region the transverse bands are closely approximated, but to avoid confusion they are shown separated (see Text-fig. 18).

The dotted line indicates the area occupied by the spinous portion of the intima of the rectal valve.

Text-fig. 15. Semi-diagrammatic view of the (spread out) inner surface of the region of the rectal valve. *a*. Spineless intima of the hind-gut. *b*. Proximal portion of the valve region, provided with very numerous distally directed spines, which are longest proximally. Distally this spine-bearing intima encircles the gut. *c*. Striated portion. *d*. Pavement portion. *e*. Distal portion, exhibiting proximally an area of spineless plates, and then areas with plates showing progressively dentations, fine comb-like spines, strong single spines and finally traces of spines.

In the insets I, II, III, IV enlargements of spines from selected areas are shown together with diagrammatic longitudinal sections of the spines and the structures in connection with them.

1. Spine. *2*. Opalescent layer. *3*. Fibrilla in opalescent layer. *4*. Cell. *5*. Muscle layer. *6*. Intercellular space.

Text-fig. 16. The changes which occur when the rectal valve passes from the closed (I) to the open (VI) position are illustrated in I–VI. In the normal position (I) the valve is kept closed by tonic contraction of the muscle of the "proximal portion" of the valve region. The opening of the valve seems to be brought about by a wave of reverse peristalsis before which the bulge in the musculature accommodating the valve retreats (II–VI). As the wave advances the "pavement portion" of the intima becomes inverted and the "striated portion" everted, owing to the restricted range of movement allowed to the intima by the fixed positions of the outer ends of its underlying cells. The constant position of the area of attachment (*g*) to the sheath of the muscle layer of the cells composing the "shelf" is indicated by arrows. The peritrophic membrane has been omitted, but its position is indicated by dotted lines in II and IV. VII. The "region of the rectal valve" in *P. groenlandica* as seen by transmitted light in a fresh preparation immersed in salt solution. VIII. The same as seen by reflected light on a dark background. IX. The "region of the rectal valve" (*Calliphora erythrocephala*)

laid open to exhibit its inner and cut surfaces. X. A portion of the distal surface of the dome formed by the pavement portion in the closed position of the valve (*Calliphora erythrocephala*).

a. Muscular coat. *b*. Lumen of the "distal portion." *c*. Spines of the "distal portion." *d*. Spines of the "pavement portion." *e*. "Striated portions." *f*. "Shelf." *g*. Region of the attachment to the muscle sheath of the cells forming the shelf. *h*. Lumen of the "proximal portion." *i*. Spines of the "proximal portion." *j*. Opalescent layer of the "valve" with fibrillae. *k*. Junction of the "distal" and "pavement" portions. *l*. Junction of the "striated" and "proximal" portions. *m*. Cells of the "proximal portion." *n*. Opalescent layer of the "proximal portion." *o*. First part of the rectum. *p*. Dome formed by the "pavement portion" in the closed position of the valve. *q*. Opalescent layer of the "distal portion." *r*. Muscular coat of hind-gut. *s*. Spineless intima of the hind-gut. *t*. Lumen of the hind-gut. *u*. Subsidiary folds on the inner surface of the "striated portion."

Text-fig. 17. I. Internal surface of the distal two-thirds of the "region of the rectal valve" spread out after maceration and removal of the intima. II. Unopened intima of the "region of the rectal valve" after maceration and removal of the muscular coat. The specimen retains the folds found in the closed position of the valve. III. The same specimen showing the folds obliterated by traction on its ends.

a. First part of the rectum. *b*. Distal portion of the rectal valve region. *c*. Pavement portion. *d*. Apparent depression in the opalescent layer. *e*. Striated portion. *f*. Opalescent layer. *g*. Proximal portion of the valve region. *h*. "Shelf" formed by cells extending into the apparent depression in the opalescent layer.

In the upper half of I the usual appearance of such specimens is shown, the cells remaining close together. In the lower half the cells have been partially separated by teasing. In III some of the folds in the striated region and its crenated proximal and distal margins are indicated.

Text-fig. 18. Semi-diagrammatic illustration of the side view of the rectal pouch to show the arrangement of the external transverse and internal longitudinal (dark) muscle bands. At the proximal (left) end the transverse bands in the terminal region of the first part of the rectum are closely arranged forming a sphincter. The transverse bands of the pouch which pass near the bases of the papillae are deflected and inserted into the margins of the radial membranes in such a manner as to produce radial traction (see Pl. VII, fig. 8; Text-fig. 19). Those which pass between the bases of the two papillae break up into branches which are inserted into similar branches of bands from the opposite side. The longitudinal bands originate as lateral, distally directed branches of the transverse bands forming the sphincter (see Text-fig. 14). Those situated dorsally and ventrally pass without interruption to the anal region of the rectum. Those situated on the sides, however, break up into branches, which give rise to bands encircling the bases of the papillae.

Text-fig. 19. The proximal half of the base of a proximal rectal papilla showing the insertions of the outer transverse muscle bands on the left and of the inner longitudinal bands on the right.

a. Outer transverse muscle bands. *b*. Inner longitudinal muscle bands, modified to form circular bands round the base of the papilla. *c*. Outer margin of the radial membrane into which the muscles are inserted. *d*. Nucleus of a radial fibre. *e*. Depression in the radial membrane accompanying a radial fibre. *f*. Convex portion of the radial membrane between depressions. *g*. Inner margin of the radial membrane, where it dips down into the papilla to become the infundibular membrane. *h*. Semilunar foramen. *i*. A cortical trachea passing through the infundibular membrane to enter the intercellular sinus. *j*. Upper part of the medullary sheath with numerous nuclei. *k*. Medullary tracheae, passing down into the medulla and giving off cortical tracheae. *l*. Upper portion of the infundibular membrane *m*. Radial membrane.

Text-fig. 20. Diagram showing the structure of a papilla in longitudinal section. On the left side the structures are shown in their normal relations, but on the right the subcellular space has been opened by drawing the cortex away from the calyx. Consequently, on the right the grooved abdominal surfaces of the basal cells, the hexagonal inner surfaces of the median cells and the rounded inner surfaces of the apical cells of the cellular layer are visible on the

outer wall of the subcellular space through the thin subcellular membrane (*j*). Also on the right side the intercellular sinus (*y*) is represented as completely laid open. Pairs of broken lines across the sinus represent the cell boundaries and the spaces between these lines passages into the next longitudinal section of the sinus. A tracheal branch (*x'*) is shown passing through one of these passages. Since longitudinally the line of junction of the hexagonal cells is angulated, the tracheae within the sinus are represented as following such a course. The approximate areas of contact of the cells on each side of the sinus are represented by stippling. Further, on the right side the trabeculae passing between the infundibular membrane and the medullary sheath have been removed in order to demonstrate the infundibular space more clearly. Arrows indicate the continuity of the intercellular sinus, through the infundibular space, with the abdominal cavity. The upper arrow is passing through a semilunar foramen. Within the medulla the medullary openings of the transinfundibular canals and two apical tracheoles arising from a medullary trachea are shown on the left side, while on the right side are shown the distribution of the terminal tracheoles arising from a medullary trachea and nuclei of the cells lining the medullary sheath.

a. A large branch of the papillary trachea entering the base. It gives off two *lateral branches*. The right lateral branch (*v*) subdivides into four *cortical branches*, which pierce the infundibular membrane (*k*). One of these (*v'*) is traced into the intercellular sinus (*y*), where it divides into two branches which pass down the whole length of the sinus. The inner branch is represented as giving off small internal lateral branches which, passing between the central cells on to their inner surfaces, terminate in tracheoles (*x*) which penetrate the cells. The outer branch is represented as giving off a small branch (*x'*) which traverses the space between two of the central cells into an adjacent section of the sinus. After giving off the lateral branches the trachea is continued as a *medullary trachea* (*w*) in the medulla, where it is represented as dividing into two branches, both of which break up suddenly into smaller branches without rings. The branch on the left is shown giving off amongst others (cut short) two *apical tracheoles* which leave the medulla and passing through the infundibular space supply the apical part of the intercellular sinus. Tracheoles supplying the medulla are seen radiating from the branch on the right. *b*. *Radial membrane*. The nuclei of the *radial fibres* are seen in the depressions between the folds. *c*. Line at which the radial membrane dips downwards to become the infundibular membrane. *d*. Muscle layers inserted into the margin of the radial membrane at the base of the papilla. *e*. *Sub-intimal cells*. *f*. *Intima*. *g*. *Cells of the cellular layer* in longitudinal section. The spaces between the cells are communications between longitudinal portions of the intercellular sinus. *h*. *Subcellular space*. *h'*. *Subcellular space* opened out. *i*. External sheath. *j*. *Subcellular membrane*; on the left in section, on the right spreading over the inner surfaces of the cells of the cellular layer. *k*. *Infundibular membrane*. *l*. *Medullary sheath*. *m*. Arrow passed through a *transinfundibular canal*, connecting the medullary and subcellular spaces. The wall of the *trabecula* in which the canal is situated is shown on either side of the arrow. Six other trabeculae and their canals are shown above and two below. *n*. Inner opening of transinfundibular canal. *o*. *Infundibular space* (on the left side apparently interrupted by the trabeculae). *p*. Lower, narrow part of the infundibular space into which the medulla does not extend, but through which the apical tracheoles of the medullary system pass. *q*. *Apical tracheoles*. *r*. Junction of the infundibular and subcellular membranes. *s*. Expanded apical portion of the intercellular sinus, into which open the lateral portions of the sinus (*y*) and the lower part of the infundibular space (*p*). *t*. Spines of the external sheath. *u*. Grooved abdominal surfaces of three of the basal cells underlying the radial membrane. *v*. *Lateral branch* of the trachea. *v'*. *Cortical branch* of the trachea in the intercellular sinus. *w*. *Medullary branch* of the trachea. *x*. Tracheoles passing into the cells of the cellular layer. *x'*. Tracheal branch. *y*. Intercellular sinus. *z*. Nucleus of a medullary cell.

Text-fig. 21. Diagram representing a dissection of a segment of the base of a rectal papilla in which the layers, commencing internally, have been removed successively from right to left.

A. *Upper part of the medulla*. *a*. A large branch of the papillary trachea entering the papilla and giving off a *lateral branch* (*b*), and a *medullary branch* (*c*), which supplies the medulla. The lateral branch divides into several *cortical branches* (*b'*), which pass through

the infundibular membrane (*n*) into the intercellular space (see Text-fig. 20). *d*. One of the *semilunar foramina*, affording entrance from the abdominal cavity into the infundibular space. *e*. A portion of the *medullary sheath*. This portion of the sheath stretches between the cortical and lateral tracheae and separates the basal part of the medulla from the abdominal cavity (see Text-fig. 19). *f*. Interior of the *medulla*. *g*. Inner or medullary opening of one of the transinfundibular canals. Folds in the medullary sheath round the orifice of the canal give this opening a star-shaped appearance. *h*. Nucleus of one of the cells lining the medullary sheath. (To avoid complicating the diagram the bodies of the cells have been omitted. See Text-fig. 22.) *i*. A tracheole supplying the upper part of the medulla. *j*. Vertical portion of the medullary sheath in section. This sheath forms the outer wall of the medulla and the inner wall of the infundibular space. *1*. Arrow passing through a *semilunar foramen* and reappearing in the infundibular space below the horizontally cut margin of the lower part of the medullary sheath. *2*. Arrow indicating the path of fluid as it passes along the infundibular space avoiding the trabeculae. *3*. Arrow passed through a *transinfundibular canal* in a *trabecula* (represented in longitudinal section) to show how these canals connect the space within the medulla and the subcellular space. *4*. Arrow passed through the canal in an intact trabecula. The points of arrows *3* and *4* lie in the subcellular space and their feathered extremities in the space in the medulla.

B. The *radial membrane* and its extensions. *k*. Sheath on the abdominal surface of the muscular layer apparently continuous with the radial membrane. *l*. The thick *radial membrane* extending from the line of insertion (*q*) into it of the muscle layer to the line (*m*) where it dips sharply downwards to become the *infundibular membrane* (*n*), forming the outer wall of the infundibular space. The greater part of the radial membrane is thrown into radially arranged folds. The radial fibres lie beneath the depressed areas. *o*. Outer or subcellular opening in the infundibular membrane of one of the transinfundibular canals. Folds in the membrane round the orifice of the canal give the opening a star-shaped appearance.

C. *Subcellular membrane*. A very thin membrane covering the inner surfaces of the cells, smooth where it covers the central cells, whose outlines can be seen through it (*s*), and folded where it covers the grooves in the basal cells. It seems to be continuous with the basement membrane of the sub-intimal cells. The *radial fibres* (*r*) appear to lie between it and the radial membrane, and to be inserted into the latter along the lines *q* and *m*.

D. *Cellular layer*. *t*. Sub-intimal cells of the pouch wall. *u*. A basal cell of the papilla showing its grooved abdominal surface, its inner and one lateral surface (vertical shading). The abdominal and inner surfaces of two adjacent basal cells are also shown. Below these are indicated the hexagonal inner surfaces of several central cells, and the lateral surfaces of three of them.

E. *External sheath*. *v*. Crenated margin of the external sheath. *w*. Intima of the pouch continuous with the external sheath (*x*). *y*. A spine on the external surface of the sheath seen through it. *z*. Longitudinal grooves in the external sheath.

Text-fig. 22. On the left is shown a diagrammatic transverse section through half a rectal papilla (*Calliphora erythrocephala*), and on the right a diagrammatic longitudinal section through part of the medulla. A. Cortex, consisting of the external sheath, cells of the cellular layer and subcellular membrane. B. Calyx, consisting of the infundibular membrane and space, the medullary sheath and medulla.

a. Medullary trachea. *b*. Medullary space. *c*. Chitinous investment supporting medullary tracheoles. *d*. A portion of the intercellular sinus, showing two longitudinal trunks of cortical tracheae in transverse section within it. *e*. A lateral branch of one of these trunks passing on to the internal surface of the cells, where it breaks up into branches terminating in intracellular tracheoles. *f*. Nucleus of a cell of the cellular layer. *g*. An intracellular tracheole. *h*. Medullary sheath (with cells lining it). *i*. Subcellular membrane. *j*. Infundibular membrane. *k*. The arrow points to a transinfundibular canal. *l*. Infundibular cavity. *m*. Subcellular space. *n*. A branch of a cortical trachea passing from one longitudinal portion of the intercellular sinus to another. *o*. External sheath showing grooves (in transverse section) and spines. *p*. A nucleus of a cell of the tracheal epithelium. *q*. A cell lining the investment of a medullary trachea.

Text-fig. 23. Diagram illustrating the branches of a medullary trachea.

a. Infundibular membrane. *b*. Infundibular space, with arrows indicating the probable direction of the flow of fluid. *c*. Medullary sheath. *d*. Inner wall of medullary cavity (stippled). The cells and the inner orifices of the transinfundibular canals have been omitted. *e*. One of the medullary tracheae breaking up into seven intramedullary tracheoles and two apical tracheoles. *f*. Subcellular space. *g*. Subcellular membrane. *h*. Arrow passing from the medullary space through a transinfundibular canal into the subcellular space. *i*. One of the intramedullary tracheoles passing towards the medullary sheath to divide into branches which ramify on the wall of the medulla. *j*. Two apical tracheoles passing down within the medulla. *k*. Apical portion of the medulla. *l*. Lower part of the infundibular space with the two apical tracheoles running in it. *m*. Junction of the infundibular and subcellular membranes. *n*. The apical tracheoles diverging in the sac-like dilatation of the intercellular sinus to pass upwards and supply the lower portions of the sinus (see Text-fig. 20).

Text-fig. 24. A. Adaptation to *Calliphora erythrocephala* of Wigglesworth's (1932) diagram illustrating his suggestions as to the course of the water circulation in the alimentary canal and excretory system.

a. Mid-gut. *b*. Peritrophic membrane (represented as broken up in the rectal pouch). *c*. Hind-gut. *d*. Rectal valve. *e*. First part of rectum. *f*. One of the rectal papillae. *g*. Rectal pouch. *h*. Semi-solid faeces in the anal portion of the rectum. *i*. Anus. *j*. Duct of two Malpighian tubes. *k*. Malpighian tube, secreting portion. *l*. Integument of abdomen. The arrows indicate the course of the suggested water circulation. "Fluid is probably both secreted and absorbed in the hind-gut, especially in the rectum, and returned to the gut by the Malpighian tubes."

B. The orifice of a transinfundibular canal. *1*. One of the folds in the membrane (infundibular membrane or medullary sheath) leading to the lumen (*2*) of the canal.

C. Rectal papilla turned "inside out" by traction on the radial membrane after removing the basal cells of the cellular layer, exhibiting the continuity of the infundibular membrane (*5*) with the subcellular membrane (*6*) and demonstrating the walls of the subcellular space. *3*. Radial membrane. *4*. Medulla (dotted) within the medullary membrane. *5*. Constricted lower portion of the infundibular membrane. *6*. Subcellular membrane, with the inner surfaces of the cells visible through it.

D. Rectal papilla as seen when placed in weak potash solution. *7*. Muscles of the pouch. *8*. Intima of pouch. *9*. External sheath. *10*. Cellular layer, surrounding the calyx.

E. Early stage in the separation of the external sheath.

F. Later stage.

G. Complete separation of the sheath from the other tissues of the papilla (see p. 232).

H. A group of spines, superficial and side views, from the apical region of the external sheath of a rectal papilla.

EXPLANATION OF PLATES V–IX

PLATE V

Fig. 1. Musculature of the crop-duct. ×120. (See Text-fig. 3.)

Fig. 2. Muscles (external) of the proventriculus (below) and of the thoracic ventriculus (above). ×80. (See Text-fig. 8.)

Fig. 3. A portion of the crop wall, showing parts of four narrow longitudinal muscle bands. Between them are seen communications of their sheaths, some of which transmit muscle fibres. ×300. (See Text-fig. 6 B.)

Fig. 4. Another portion of the crop wall, showing parts of five narrow parallel muscle bands, less highly magnified. The ill-defined round bodies between the bands are nuclei of the subintimal cells. ×180.

Figs. 5 and 6. Portions of the crop wall, showing in both cases narrow parallel muscle bands above and broad bands below with the "intervening network" between. ×75.

PLATE VI

Fig. 1. A segment of the middle portion of the thoracic ventriculus, showing six transverse muscle bands with their dorsal and ventral decussations bounding lateral spaces through which the inner coats project to form the external transverse folds. The longitudinal bands are visible as thin lines. ×50.

Fig. 2. The distal end of a Malpighian duct (below), showing transverse and longitudinal muscles, with the proximal ends of two tubes (above), without muscles, opening into it. ×100. (See Text-fig. 12.)

Fig. 3. Enlargement of three of the dorsal decussations in the transverse bands shown in Fig. 1. In each transverse band above the decussation and in each below a nucleus is visible, situated in a cloudy area near the upper or lower margin of the field respectively. The longitudinal bands are seen crossing the transverse bands, two across the upper nuclear region, one of which branches, six across the decussation and two, both showing branching, across the lower nuclear region. ×200.

Fig. 4. Inner surface of the aorta laid open to show the very minute transverse and longitudinal muscle bands. The junction of two "sections" is seen in the middle of the figure. ×120.

Fig. 5. A segment of the abdominal ventriculus showing broad transverse bands, defined by the narrow intervening spaces, and numerous thin longitudinal muscle bands, some of which, especially on the left, are branched. ×110.

Fig. 6. A segment of the hind-gut showing broad transverse muscle bands often terminating in expansions. Very numerous longitudinally disposed lateral branches are visible. ×80. (See Text-fig. 13, *h*.)

Fig. 7. Junction of the mid- and hind-gut. ×75. (See Text-fig. 13.)
The arrows point to the long axis of the gut.

PLATE VII

Fig. 1. Longitudinal section of the rectal valve in the "closed position." The long spines of its wall are seen projecting into the lumen of the "proximal portion," which is filled with the folded peritrophic membrane. ×250. (See Text-fig. 16 I.)

Fig. 2. Rectal valve in the "closed position" in optical section. ×50.

Fig. 3. Rectal valve in the "open position" in optical section. The arrow points to the "shelf," which is clearly visible. ×50.

Fig. 4. Longitudinal section in the "striated region" of the rectal valve, showing elongated cells passing from the outer wall (right) to the opalescent layer (left). ×500. (See Text-fig. 15 II.)

Fig. 5. Longitudinal section in the "proximal region" of the rectal valve, showing an elongated cell (arrow) passing from the outer wall (right) to the base of one of the long spines (left). ×400. (See Text-fig. 15 I.)

Fig. 6. Tracheae passing down basal third of medulla. ×600. (See Text-fig. 22.)

Fig. 7. Side view of the rectal pouch, showing on the left the distal sphincter (arrow) of the first part of the rectum with the origins of the longitudinal muscle bands of the pouch, and on the right the distribution of the transverse (broad) and longitudinal (narrow) bands of the pouch in relation to the proximal rectal papilla. ×70. (See Text-fig. 18.)

Fig. 8. The insertion of the muscle bands of the pouch into the radial membrane (centre). The radial folds in the latter and some of the nuclei of the radial fibres are well demonstrated. The calyx (black) is attached to the centre of the radial membrane. The black line crossing the specimen is the nerve, which supplies a branch to the papilla. ×90. (See Text-fig. 19.)

Fig. 9. Side view of the calyx, showing its shape, its attachment to the radial membrane (arrow) and the medullary tracheae passing into it. ×90. (See Text-fig. 20.)

Fig. 10. Calyx attached to the radial membrane. Towards its apex the trabeculae (as small projections) and the infundibular membrane are just recognisable. ×80.

PLATE VIII

Fig. 1. A portion of the radial membrane spread out to show its continuity with the infundibular membrane, which is seen below on the left. The numerous dark punctiform marks on the latter are the outer orifices of the transinfundibular canals. (The medullary sheath, medulla and cortical trachea have been dissected off.) ×125. (See Text-fig. 21 B, *l*, *m*, *n*.)

Fig. 2. A portion of the internal margin of the radial membrane and of the basal surface of the medulla in an intact papilla, showing (above) the radial membrane covering the basal cells and dipping down along a sharp curved line to become the infundibular membrane, and (below) the cortical tracheae united by the basal layer of the medullary sheath, except near the radial membrane where semilunar foramina are left between the cortical tracheae. ×500. (See Text-fig. 19, *f*, *g*, *h*, *i*, *j*.)

Fig. 3. Inner surfaces of some of the central cells of the cellular layer. ×250. (See Text-fig. 21 D.)

Fig. 4. Side view of a rectal papilla in which the apical portion of the intercellular sinus and infundibular space, surrounding the medulla, have been injected with graphite. ×60. (See p. 231.)

Fig. 5. A more highly magnified view of the apex of the papilla illustrated in Fig. 4 to show the relationship of the graphite in the branches of the intercellular sinus to the tracheae (dark lines in the upper part of the figure) running in these channels. ×180.

Fig. 6. The rectal valve in the "closed position" in a fly which had been fed on Indian ink in syrup. The central thin line (arrow) is due to small quantities of Indian ink remaining in the constricted part of the peritrophic membrane, which occupies the lumen of the closed valve. Above and below the valve the lumen is large and contains Indian ink. ×50.

Fig. 7. The inner surfaces of two cells (in the centre) of the cellular layer. They are surrounded by tracheae lying in indistinct branches of the intercellular sinus. Tracheoles (fine lines), springing from these tracheae, course over their surfaces. ×*c*. 500.

Fig. 8. The same cells focussed at a point between the inner surface and the nucleus. Different portions of the surrounding tracheae are now in focus, and the tracheae are seen to be running in clear zones, which are portions of the intercellular sinus surrounding the cells. The portions of the tracheoles now in focus are intracellular.

Fig. 9. The same cells focussed at the level of the nuclei. The portions of the intercellular sinus are still better defined, but the tracheae within them have almost passed out of focus. A few intracellular tracheoles are still visible. (See Text-fig. 22.)

Fig. 10. A portion of the external chitinous sheath of a papilla showing spines and grooves. (See Text-fig. 21 E.)

Fig. 11. A portion of a radial membrane with the nuclei of the radial fibres rounded and close to the periphery of the membrane. ×150. (See p. 225.)

Fig. 12. Longitudinal section of a rectal papilla in which the subcellular space is distended and contains a granular coagulum (arrows). The infundibular membrane and the medullary sheath, with trabeculae stretching between them, are clearly visible surrounding the medulla. ×40. (See p. 224.)

Fig. 13. Cells of the cellular layer separated by the removal of the subcellular membrane. ×250. (See p. 222.)

PLATE IX

Fig. I. Dissection of the alimentary canal of a blow-fly killed immediately after ceasing to feed on liquid human faeces, showing all parts distended with the food. In several places the position of the peritrophic membrane within the gut can be recognised. ×6.

Fig. II. Dissection of the alimentary canal of a blow-fly from the proventriculus to the anus killed immediately after ceasing to feed on liquid human faeces. In two places the wall of the abdominal ventriculus has been opened and loops of peritrophic membrane (*l*, *l'*), into which most of the food in the region has been forced by muscular contraction, have protruded. ×6.

Fig. III. Dissection of the alimentary canal of an unfed blow-fly from the proventriculus to the anus, with the wall of the abdominal ventriculus removed as far as the helicoid portion in order to demonstrate the peritrophic membrane (*m*, *m'*) in the empty condition. ×6.

a. Proboscis. *b*. Oesophagus. *c*. Crop-duct. *d*. Crop. *e*. Proventriculus. *f*. Thoracic ventriculus. *g*. Abdominal ventriculus. *h*. Helicoid region. *i*. Hind-gut. *j*. Rectal valve. *k*. Rectal pouch. *l*, *l'*. Loops of distended peritrophic membrane. *m*, *m'*. Empty peritrophic membrane.

For these photographs I am indebted to the care and skill of Mr H. P. Hudson, of the Pathological Department, Cambridge.

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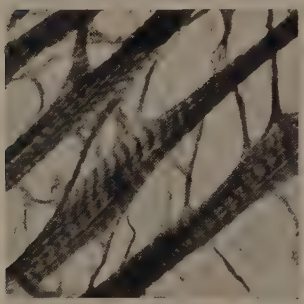

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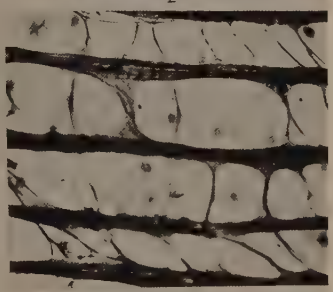

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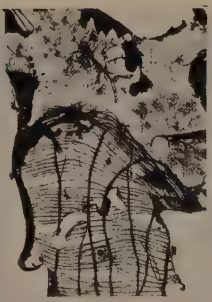

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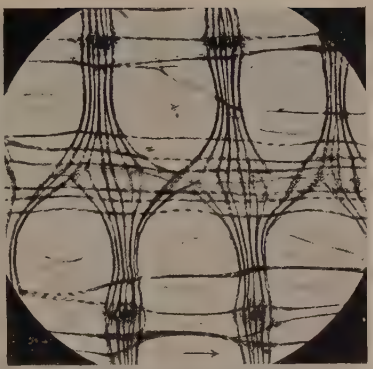

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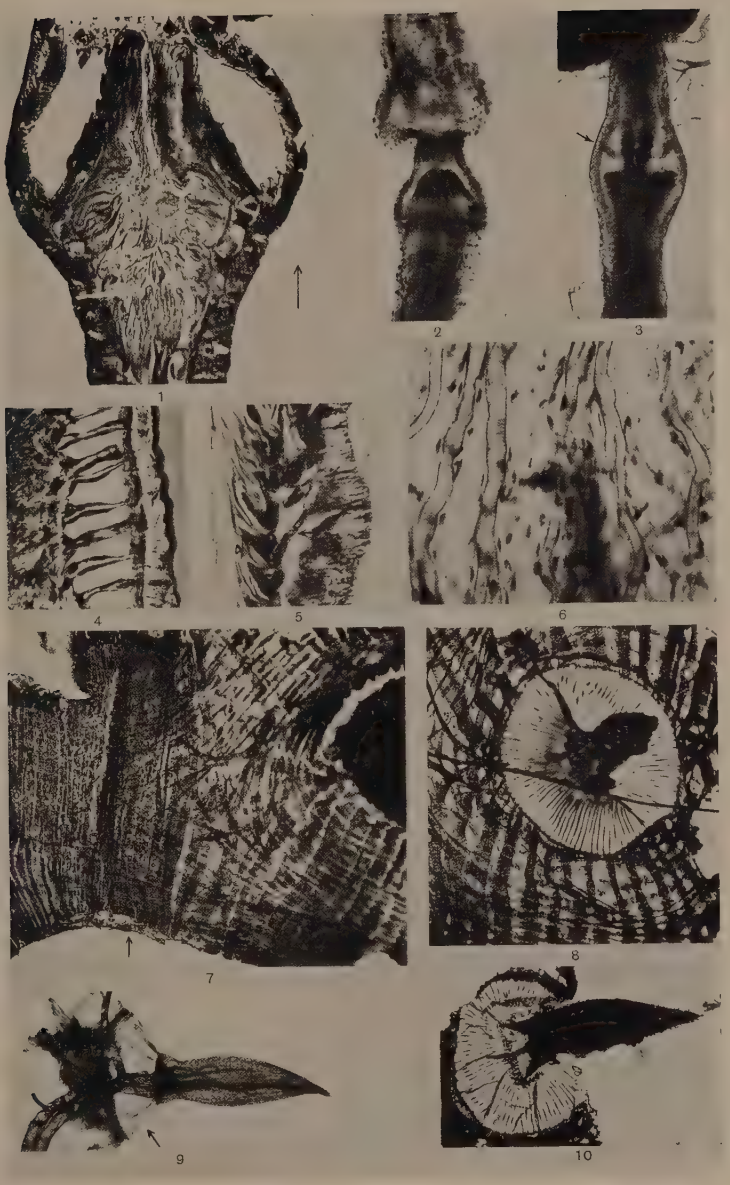
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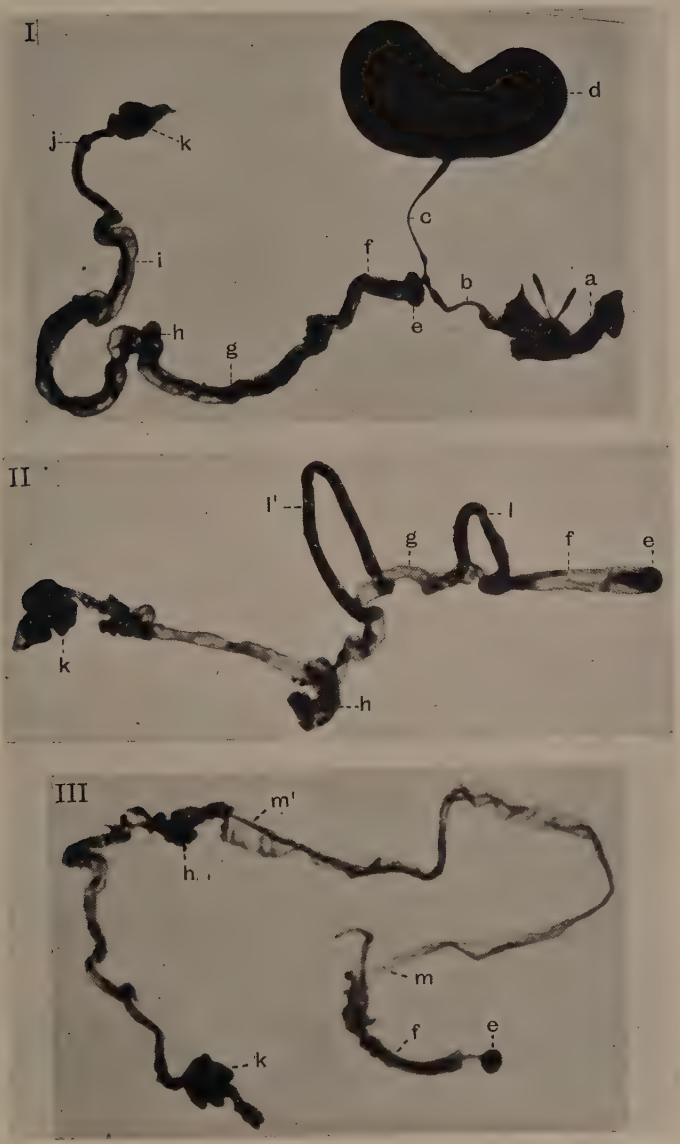
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FURTHER STUDIES ON THE BIOLOGY OF THE PIGEON FLY, *PSEUDOLYNCHIA MAURA* BIGOT (DIPTERA HIPPOBOSCIDAE)

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Introduction

In a previous contribution by the junior author (Coatney, 1931), attention was called to certain points in the biology of the pigeon fly which heretofore had not been recorded. Since the knowledge of this group of ecto-parasites is very incomplete a continued study seemed warranted. The present paper attempts to record additional points in the biology of this fly with special emphasis on the tropisms.

The authors wish to express their thanks to Dr F. C. Bishopp, of the Bureau of Entomology, United States Department of Agriculture, for his kindness in supplying the initial stock of flies for these experiments.

Mating and larvaposition

The mating habits of this insect have not been described in detail although Coatney states that "mating generally begins while the flies are on the host. The female spreads her wings widely, then the male holds on to her neck with his forelegs and bends his abdomen ventrally to insert the penis." In order to add to these observations the sexes were separated as they emerged and put together after 12, 36 and 72 hours. In no case did copulation take place until the third day, and only then if the male had not recently fed. The male mounts the female from the side with a swift running motion. His forelegs are clasped around the female just posterior to the head, and the hooks on his tarsi are sunk into the fleshy triangle formed by the female's two front legs and the ventral chitinous plate. The second pair of legs clasp the female at the junction of the thorax and abdomen. Her wings are separated widely by his third pair of legs, which are then projected posteriorly. The entire process of mounting and separating the wings is so swift as to prohibit observing the exact sequence of events, but it is thought to be almost simultaneous. The abdomen of the male is now strongly flexed ventrally and closely applied to the body of the female, to allow the insertion of the intromittent organ. During copulation the female is sometimes seen to stroke the abdomen of the male with the third pair of legs. The pair remain united for a variable time, anywhere from a few minutes to 2 hours, when the male suddenly dismounts. When the female takes the initiative in severing contact

she will be seen to jerk from side to side. If this fails, she raises herself on her first and second pair of legs, and dislodges the male by pushing on his abdomen with her third pair of legs.

The insect is viviparous, the female depositing a mature whitish larva, which soon changes to a shining black (true pupa), singly in darkened corners or crevices. This habit of seeking darkened places makes the observation of the process rather difficult, but in cases where we have been able to observe larvaposition it has been noted that the female supports herself with the first two pairs of legs. One of the third pair of legs is placed on the dorsal side of the abdomen, the other on the ventral, and with a "milking" motion she strokes the abdomen as if helping the larva through the vaginal orifice.

In emerging, 25–31 days later, depending on the conditions of temperature and humidity, the young imago pushes open the anterior end of the puparium, which splits along a definite line, exposing the head and forelegs. By reaching out with the forelegs and grasping a rough surface, the imago gradually drags itself free. As the young insect comes out the second and third pair of legs are seen tightly pressed against the abdomen. Within a few minutes, after the splitting of the pupal case, the fly is able to run rapidly and the wings have filled with air.

Tropisms

Up to the present time experiments on the tropisms of the pigeon fly have not been recorded. Bishopp (1929) states that *Pseudolynchia maura* is "strongly positively phototropic" when off the host, but the reverse is true while it is on the pigeons. Freund and Stolz (1928), in their study of *Melophagus ovinus*, a closely related insect, found that this species was indifferent to oil of thyme, and oil of cloves. The oil of origanum caused convulsive cramps and, as the cramping became worse, the auxiliary hearts stopped and death ensued in 10–30 min. Rivnay (1932) determined the tropisms of the bed bug, *Cimex lectularius*, and found the insect to be positively thermotropic and stereotropic; negatively hydrotropic, and neutrally phototropic. According to Rivnay (1932), Kemper tested the responses of bed bugs to the odours of thirty-seven chemicals and found that none of those tested attracted the bugs, but that the bugs perceived and evaded the odour of some fluids such as xylol, yet were indifferent to others. As described in a previous paper (Coatney, 1931) the pigeon fly refused to feed on man when the skin was giving off perspiration.

Thermotropism

In order to test the effect of temperature on this insect flies were placed in a glass cylinder, 20×5 cm., a grid was attached at one end and kept at a temperature of 104° F. (the approximate body temperature of the pigeon); the other end was closed by a thin cork and kept cold by packing the outside with a mixture of salt and ice.

Flies placed on the heated grid remained in contact with the warm surface

even though the cylinder was violently shaken. If the flies were placed in the cold zone and the cylinder was held still and level, the flies always moved to the warm portion, usually by flying. When released in the middle zone, the insects always moved toward the warm end, but never toward the cold. When they were placed in the cold end of the cylinder, and the apparatus was held at an angle which prevented them from reaching the grid, the insects made violent efforts to reach the warm end of the tube until they were overcome by the cold. When they were made to stay in the cold end of the cylinder, they became stiff and unable to move; but when moved into the middle zone, they revived and moved toward the heated end. When within the last 2 cm. they usually flew upon the grid. If the temperature was raised to 110° F., the flies approached the grid but made no attempt to alight. In order to further test the effect of temperature on these insects a bent glass tube, closed by a fresh membrane, was passed through a hole in a litre beaker and fastened so that the membrane was flush with the inside. The tube was filled with water and kept at approximately 104° F. during the experiment. The beaker was covered with a glass plate and the flies introduced. The insects immediately settled on the bottom of the beaker. When the vessel was placed in cold water the flies became restless and made short flights in the air. One by one they were attracted to the warm membrane where as many as four or five would rest at a given time. Usually they were pushed off by other flies attempting to alight on the membrane. When pushed off, they soon returned to the warm surface.

Phototropism

Before beginning the experiments on light it had been noticed that flies accidentally released were generally recovered from dark or poorly lighted portions of the laboratory. With this in mind it was decided to carry out controlled experiments using both the starved and recently fed flies.

A series of starved flies were released individually for a total of 100 times; their flight and place of rest being carefully noted (see Table I).

Table I. *Showing places of rest of starved and recently fed flies when released individually in the laboratory.*

Places of rest	Starved	Recently fed
Light surfaces in the light	10	15
Dark surfaces in the light	12	11
Light surfaces in the dark	47	41
Dark surfaces in the dark	29	31
Light surfaces in the light (later changing to dark)*	2	2

* Flies immediately moved into the dark zone after first coming to rest in the light area.

The flies used in the experiment were allowed to feed on a pigeon and then released and caught for a total of 100 times as given above (see Table I). These flies were very sluggish and slow in their movements but, as may be

seen from a study of the table, the results closely follow those obtained for the starved flies.

Thigmotropism

From observations made in infected cotes, it was evident that rather tight cracks were preferred as places to deposit pupae, but whether this was a negative response to light or a positive thigmotropism was not known. Scores of flies placed in cotton-plugged vials in the laboratory were found with their heads and thoraces firmly pushed in between the cotton and the glass of the vial. Even in those vials stoppered with cork or rubber the flies were found trying to push their way into the small cracks. In glass jars with cotton pads on the bottom and with illumination from beneath, the flies were found congregated with heads and thoraces, or even whole bodies, under the pads. Flies with clipped wings released on a table immediately pushed under the edge of a sheet of paper and then remained quiet.

Hydrotropism

When flies were placed in a covered glass jar having a raised centre and water in the depressed portion, they sooner or later came into contact with the water and then immediately withdrew to the dry portion of the jar. When forced into the water they made violent efforts to regain the dry surface. When this was prevented, they raised themselves from the water by crawling on one another. When warm water was placed in the jar, the flies were attracted to the water's edge, but retreated after the forelegs came in contact with the moisture.

Chemotropism

The experiments in chemotaxis were undertaken to discover if an odour provoked a response. A positive reaction is designated as one in which the fly moves towards the source of the stimulus; if it withdraws, the reaction is considered negative. The term indifferent is used when no response is noted. The odour of solids was tested by placing a small portion of the material at one end of a glass cylinder, 16×5 cm., in which the flies were confined. A few drops of the liquid to be tested were placed on a bit of cotton attached to the inside of the stopper. All experiments were carried out on starved flies in an artificially lighted laboratory at room temperature.

Fresh blood. Indifferent.

Fresh pigeon skin. Indifferent.

Pigeon scales and feathers. Indifferent.

Ammonia. Indifferent. Since fresh pigeon droppings contain appreciable amounts of ammonia, different percentages (0·1, 0·5, 1·0, 2·0, 4·0) of this reagent were tried.

Oil of cloves. Positive. All were attracted to this oil, and alighted on the soaked cotton. Because of the moist condition they left at once. The flies remained in the immediate vicinity of the oil as long as it was present.

Oil of wintergreen. Negative. Convulsive cramps took place 10 min. after exposing the insects to the reagent. Death ensued to those within 7 cm. The flies at a distance of 8–13 cm. recovered.

Oil of thyme. Considered indifferent. Flies in the vicinity of the oil became restless but failed to retreat. After removing the oil, alternate cramping and straightening of the legs were noted for 30 min. One specimen accidentally came in contact with the oil early in the experiment and immediately became convulsive. While in this semi-anaesthetised condition the flies were removed and placed on a pigeon, but they were unable to cling to the feathers. Recovery of all flies took place in 70–90 min.

Oil origanum. Indifferent.

Xylol. Indifferent. This reagent caused convulsive reactions within 3 min. If removed when the cramping began all the flies recovered in 25–35 min.

Camphor. Positive.

Bergamot. Indifferent.

Amyl alcohol. Negative. One specimen that came in contact with the reagent early in the experiment showed immediate convulsions. Death took place in a few minutes.

Ox gall. Positive. This reaction did not become evident for 8 min.

ATTEMPTS TO FEED ARTIFICIALLY

As shown in the foregoing experiments, the pigeon fly shows a positive response to heat, pressure and certain chemicals. Because of these facts the question that next presented itself was, can these insects be fed artificially? We were particularly anxious to answer this question since a method of artificially feeding this fly would greatly facilitate the work in progress on *Haemoproteus columbae* of the pigeon's blood.

It is recorded in the 1926 *Proceedings of the Indian Scientific Congress* that S. R. Nair verbally reported before that body an attempt to feed the pigeon fly artificially. His results were never published and therefore the data is not available. Other investigators have been successful in their attempts to artificially feed certain other blood-sucking insects; notable among these are Huff (1929), who fed *Culex pipiens* upon a variety of foods and collected viable ova, and Rivnay (1930), who succeeded in feeding the larval instars and adults of the bed bug, *C. lectularius* L.

Before beginning the experiments on artificial feeding it was decided to determine the life-span without food in order to better interpret our feeding results. Sergent (1909) states that the unfed pigeon fly lives only 2–3 days, while Adie (1915) and Hase (1927) give 48 hours. In carrying out the experiment young imagines were placed in separate vials and these containers divided into three series. Series 1 was violently shaken at short intervals with the result that the flies died after 75–77 hours. Series 2 was disturbed at longer intervals, and in this group the insects lived for 84–92 hours. The flies in series 3 were not disturbed, and lived for approximately 109 hours.

Experiments

There are several factors to be considered in attempting to feed this fly artificially. The structure of the mouthparts prevents feeding the fly on moist foods or exposed liquids. The insect will not attempt to pierce a smooth surface or one emanating a strong odour even though it radiates heat. Temperatures less than 94° F. do not attract the fly and those above 110° F. repel. Insects fewer than 12 hours old refuse to take food. In order to overcome as many of these difficulties as possible the apparatus was modified from that described under thermotropism. For this experiment, a glass cylinder (20 × 5 cm.) was used to confine the flies. One end of the cylinder was closed by a cheese cloth and the other by a thin cork stopper. The bent-glass tube was passed through a hole in the stopper and then wrapped with a resistance wire. The coil was connected to a rheostat for maintaining the proffered food at a constant temperature. The membranes used in the experiments were fresh, refrigerated, non-refrigerated and cooked pigeon skin; scraped chicken skin; turkey skin; sausage casings; prune skin; and guts of sheep, pig, pigeon, fish, chicken and dog.

Exp. 1. The feeding tube was filled with fresh defibrinated pigeon blood, using a fresh pigeon skin as a membrane. The blood was not warmed and the flies refused to approach the membrane. When the temperature of the blood was raised to 94° F. one of the insects immediately approached and placed its forelegs against the membrane. For the next 5 min. the maxillary palps slowly vibrated laterally against the warm surface. Suddenly the fly settled upon the membrane and inserted the haustellum. The insect fed for 7 min., the abdomen becoming engorged and red.

Other flies were also attracted to the membrane and vibrated their palps as described above, but did not attempt to insert the haustellum. There was a constant moving around on the membrane with occasional short flights, only to return at once to the warm surface. After the blood became cold the insects moved away and did not return.

Exp. 2. The flies were given fresh defibrinated pigeon blood, using a slightly rancid pigeon skin as a membrane. The insects were at first attracted by the heat, but upon reaching the vicinity of the membrane were actively repelled. The rancid skin was replaced by a fresh one, whereupon they settled on the surface, but did not feed.

Exp. 3. Unfed flies were given a saturated sucrose solution warmed to 106° F. The sugar solution rapidly penetrated the thin sheep-gut membrane, causing the external surface to become moist and catching the flies in the sticky material. Death ensued in a few minutes. The experiment was repeated after the gut had been dried in the oven. Approaching the membrane the flies gently stroked it with the maxillary palps. As the gut was very smooth, the stroking gradually diminished, and the insects made no attempt to insert the haustellum.

Exp. 4. A series of flies were given juice expressed from cooked raisins. When this material was warmed to 106° F. it attracted the flies at once. All insects that were observed taking food failed to survive the first feeding. Other foods, including defibrinated pigeon blood and physiological salt solution, defibrinated pigeon blood and 0·1 per cent. sucrose solution, apple juice, Karo (corn) syrup, vegetable beef soup, bovine serum, whole milk, were offered as outlined above and the results summarised in Table II.

The feeding apparatus used above was discarded, and a different method was used for the following experiments.

Exp. 5. A number of flies were placed in a small beaker, and the top was covered with cheese cloth. A section of apple was superimposed upon the cloth so that the flies could reach the cut surface. Later the apple was removed and replaced in turn with liver and pads of cotton soaked in varying concentrations of sucrose. There was no indication that any of the flies fed on these foods.

Table II. *Showing food materials used, and resulting longevity.*

Foods used	Longevity in hours
*1. Pigeon blood	84
2. Defibrinated pigeon blood + physiol. salt solution	42
3. Defibrinated pigeon blood + 0·1% sucrose solution	82
4. Karo syrup solution	84
*5. Raisin juice	2
*6. Whole milk	117
*7. Bovine serum	91
8. Raw apple	—
*9. Saturated sucrose solution	108
10. 0·1% sucrose solution	104
11. Vegetable beef soup	72
12. Raw liver	—
13. Apple juice	—

* Foods the flies were actually seen to take.

Twenty-five flies were used in experiments 1 to 9, and ten flies were used in experiments 10 to 13.

GENERAL DISCUSSION

The act of copulation has never been fully described for *Pseudolynchia maura*. From observations made on caged flies there is reason to believe that the male locates the female largely through tactile sensations. Many times the female has been seen to brush accidentally against a passive male, whereupon he pursues and immediately mounts. There is no indication, however, that the female takes the initiative in inviting the male to approach. When the copulating individuals were confined in small wire cages, the female usually carried the male to the perpendicular sides of the cage and there remained suspended by the first two pairs of legs, the third pair usually hanging free. The males which have recently copulated will not mate again for some time.

The literature of the subject makes no mention of the process of deposition other than that noted by Bishopp when he writes, "Some time before a pupa is deposited, the male attaches itself by its tarsi to the back of the female

and remains in this position until a pupa is deposited, when copulation takes place." It is possible that the male in this position in some way serves the process of deposition so that the stroking of the abdomen by the female is not needed. At least if the male is present on the back of the female, the stroking has not been observed.

The experiments on tropisms would indicate that heat is probably the principal attracting stimulus. Since the parasite is capable of perceiving heat from some distance, its attraction to a warm surface may guide it directly to the host. If a host is motionless, and the fly is too far removed for the heat stimulus to be perceived, the motion of flight may attract the fly. Because of the positive thermotropism, it was decided to learn if the insects would attack any warm object even though its temperature might prove injurious. From all indications this does not seem to be the case. High temperatures may attract the insect at first; but when the optimum is reached, the insect fails to advance or may retreat.

Odour seems not to act independently of heat, for the insects will not exhibit feeding reactions under the influence of odour alone, but odours unpleasant to the fly will check the insect from approaching a host under the influence of heat, and may even cause it to retreat. Assuming that the odour of the host has some attracting stimulus, the effects of feathers, scales, skin and blood were tested, but the fly remained indifferent. In order to test the olfactory sense of these insects, they were exposed to penetrating odours and volatile oils. The data show that the fly is capable of detecting odours, since they are attracted by the oil of cloves, camphor and ox gall, but may not give a negative response to a reagent capable of producing harmful effects.

The habit of depositing pupae under tight-fitting false bottoms of the nests and under the water pan can be easily explained as a positive thigmotropism. The pupae are usually found just under the edge of the sliding tray of the nest, which indicates that the fly desires to have the dorsal part of the body in contact with a solid object when she deposits her offspring. Observations on insects confined in cotton-stoppered vials in a dark portion of the laboratory showed only the head and thorax of the fly pushed in between the cotton and the glass of the vial.

From observations and feeding experiments, it became evident that the pigeon fly makes no attempt to pierce a surface which is too smooth. Usually when an object radiates heat or an attractive odour, the fly will first stroke the surface with the maxillary palps; and if the surface is dry and slightly roughened, it will attempt to insert the haustellum. If the surface is too smooth, as in the case with a taut membrane, the stroking of the maxillary palps gradually diminishes and the insect makes no attempt to insert the haustellum.

The data in Table I show that both the starved and recently fed flies do not show a complete negative phototropic response, but on the contrary will even prefer light surfaces to dark ones as long as they are in the shadow. These

findings do not agree with those of Bishopp. From observations made in the cotes, there was no indication that a dark host was preferred to a light one since these insects were found in about equal numbers on white and dark birds.

In the proximity of a host (real or artificial), positive reactions may be counterbalanced by other external stimuli. The presence of moisture is very repellent to the insect; after it has once been in contact with it, it afterwards refuses to approach the dampened area. The moisture collecting on the wings renders them incapable of flight, and collecting on the body interferes with respiratory processes through the spiracles.

The failure to live on raisin juice was at first interpreted to mean that the food had become contaminated with bacterial organisms which were fatal to the insect. In order to rule out this possibility the juice was sterilised and given under strict aseptic conditions. The material still proved fatal and only further experimentation will determine the cause.

SUMMARY

1. Observations indicate that the female uses the third pair of legs during deposition as an aid in forcing the larva through the vaginal orifice.

2. The young imago is able to free itself from the pupal case by dragging the case across a rough surface with the forelegs.

3. The act of copulation has been observed and described, including mounting, position and duration.

4. Evidence is presented to show that the insects are positively thermotropic and thigmotropic; negatively phototropic and hydrotropic.

5. The flies react positively to the odours of oil of cloves, camphor and ox gall; negatively to oil of wintergreen and amyl alcohol, and are indifferent to blood, fresh pigeon skin, pigeon scales, feathers, ammonia, oil of thyme, oil origanum, xylol and bergamot.

6. The length of life without food is found to be in direct proportion to the energy expended in motion, ranging from 77 to 109 hours.

7. The flies refuse to take unwarmed food.

8. The insect will not penetrate a perfectly smooth membrane, or one that is rancid.

9. According to data presented, *Pseudolynchia maura* will not live longer than 117 hours when artificially fed.

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SOME NEW ECTOPARASITIC TREMATODES (ONCHOCOTYLINAE) FROM THE GILLS OF AMERICAN SHARKS[1]

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(With 8 Figures in the Text)

The genus *Onchocotyle* was named by Diesing in 1850 with *O. appendiculatum* (Kuhn 1829) as type. Cerfontaine (1900) reviewed the history of the group and revised the genus. He named the subfamily Onchocotylinae, containing three genera: *Squalonchocotyle*, *Acanthonchocotyle* (subsequently regarded by Monticelli (1903) as a synonym of *Onchocotyle*) and *Rajonchocotyle*. He discarded the older generic name of *Onchocotyle*, and placed the group in the family Octocotylidae. Poche (1925) placed it in the family Polystomatidae Gamble (1896). Fuhrmann (1931) lists the group in the suborder Polyopisthocotylinea, family Onchocotylidae.

In 1926 Causey described a new species as *Onchocotyle somniosi* from the sleeper shark (*Somniosus microcephalus*), and in 1927 Ruth C. Miller named *Onchocotyle striata* from *Squalus sucklii*. Neither of these authors follows Cerfontaine's classification, but both return to the original single genus. Hughes (1928) described *Squalonchocotyle antarcticus* from *Mustelus antarcticus* and in 1931 McCallum added four new species from American waters. Both authors follow Cerfontaine's generic divisions. Guberlet (1932) adds *Squalonchocotyle catenulata* from *Mustelus laevis* and accepts the classification of Poche (1925) which includes *Squalonchocotyle* Cerfontaine, *Rajonchocotyle* Cerfontaine and *Onchocotyle* Diesing, all in the family Polystomatidae.

In addition to these genera Fuhrmann lists *Erpocotyle* Van Beneden and Hesse and *Diclybothrium* F. S. Leuckhart as belonging to this family. These are both parasites of species of *Acipenser* and, although the literature is incomplete, their generic status within this group is probably valid. A more detailed discussion of the history and classification of the group may be found in the recent work of Guberlet (1932).

The following descriptions include one new genus and three new species from sharks in American waters. The specimens were collected at Tortugas, Florida, and Beaufort, North Carolina, by Dr H. W. Manter, under whose supervision and aid this paper has been prepared.

[1] Studies from the Zoological Laboratory, the University of Nebraska, No. 176.

Heteronchocotyle hypoprioni n.g. and n.sp.

(Figs. 1 and 5)

Six specimens were collected from the gills of the yellow shark (*Hypoprion brevirostris* Poey) at Tortugas, Florida, in 1932.

Description. The body is flattened and elongate, convex dorsally in cross-section, tapering anteriorly where it bears a subterminal oral sucker with transverse, ventral opening. In addition to its muscular structure this sucker is studded with minute papillae. The oral sucker region is not sharply marked off and the extreme anterior tip of the body projects dorsally beyond the sucker as a lip-like protuberance. Posteriorly the body inserts into a flat, approximately semicircular fixation disc which bears six suckers, each of which contains a highly refractive, curved, chitinous structure from one end of which projects a sharp hook or claw. The suckers themselves are strongly muscular and bear numerous small papillae similar to those appearing on the oral sucker. These are situated for the most part on the lips or rims of the suckers. The floor of the interior of the sucker cup is corrugated by a series of sharp transverse muscular ridges. In size, the chitinous hooks vary greatly (Fig. 5). Two of them are enormously developed, a third is of less size, and the remaining three are proportionately slender and inconspicuous. If these be numbered from right to left according to their relative position upon the fixation disc, numbers two and six are the largest, number one is of intermediate size, and three, four and five are small. In one specimen, this order is reversed and reads from left to right. In addition to these structures on the fixation disc there is a short, stubby appendix which bears two deep and narrow suckers at its bifurcate, distal end. Located between these suckers is a pair of small triradiate hooklets (Fig. 5*d*).

The specimens vary from 4·5 mm. × 0·9 mm. to 6·0 mm. × 1·5 mm. When fully extended the body surface is smooth, but when contracted or flexed the surface becomes evenly wrinkled, presenting a transversely striated appearance. The accompanying table of measurements will show the relative proportions of the five whole specimens.

The oral sucker communicates directly with a small spherical pharynx. The intestine, before dividing immediately posterior to the pharynx, gives off a diverticulum which lies ventral to the pharynx. The two crura of the intestine possess lateral branches which ramify through the entire parenchyma with the exception of those portions occupied by the reproductive organs. The crura unite to form one canal which passes into the fixation disc where it ramifies extensively, one branch extending into the appendix. The pores and course of the excretory system are indiscernible in the material studied.

The ovary is located at the anterior level of the posterior third of the body. It is thick, much coiled and appears as an irregular mass. The oviduct arises from the posterior end of the ovary and proceeds anteriorly, soon expanding

Fig. 1. *Heteronchocotyle hypoprioni*, ventral view.
Fig. 2. *Squalonchocotyle ginglymostomae*, ventral view.
Fig. 3. *Squalonchocotyle microstoma*, ventral view.
Fig. 4. *Squalonchocotyle tiburonis*, ventral view.

g.p. genital pore; *p.* penis; *s.d.* sperm duct; *ut.* uterus; *v.p.* vaginal pore.

(Figs. 1 and 3 drawn to same scale.)

into a thick-walled spiral seminal receptacle. This structure is unique since, instead of being an out-pocketing from the oviduct, it appears as an enlargement of the oviduct itself. In addition to its spiral nature the syncytial walls of this organ bear shelf-like projections into the interior which possibly are of some use in impeding the backward progress of the sperm cells. The interior of the receptacle contains a tangled mass of sperm cells, through which the eggs must pass on their way to the uterus. Anterior to the seminal receptacle, the oviduct again narrows and gives off the genito-intestinal canal leading to the left crux of the intestine. A short distance beyond this point the oviduct is joined by the yolk duct. Here it turns upon itself and leads posteriorly to the level of the ootype, where it again changes direction to proceed anteriorly in the median line as the straight unconvoluted uterus which opens into the genital atrium.

The follicles of the vitellaria are small, closely compact, and fill the lateral areas nearly to the median line from the level of the pharynx to the fixation disc. Posterior to the testes, however, the follicles of the vitellaria become increasingly sparse. The openings of the vaginal canals are surrounded by circular muscles. They are located on either side of the median axis about half-way between the genital pore and the anterior level of the ovary. From them the vaginae proceed first anteriorly a very short distance and then turn to run posteriorly in the lateral areas until they open separately into the vitellosac which, in turn, narrows into a small duct before entering the oviduct. No eggs were present in any of the specimens.

The testes are fifteen to twenty-five in number and occupy the intercrural space immediately posterior to the ovary. They do not extend to the extreme posterior end of the body proper, the posterior one-fourth of the intercrural space being devoid of any part of the reproductive system. Anterior to the ovary is the highly developed vas deferens which is a long and much convoluted tube filled with sperm cells. Proceeding anteriorly from the posterior end of the vas deferens a muscular sperm duct takes a serpentine course along the right medial margin of the lateral area to about the level of the vaginal pores, where it crosses to the left side of the intercrural space and runs posteriorly to the anterior level of the vas deferens, then turns sharply forward and pursues a serpentine course in the median line to the penis. The penis is strongly muscular and opens into the genital atrium through a wide anterior pore situated in the median line about half-way between the level of the vaginal pores and the bifurcation of the intestine. The genital atrium, which is small, also receives the opening of the uterus at this level and, in turn, opens to the outside through a small median ventral pore.

Generic diagnosis of Heteronchocotyle. Onchocotylinae with fixation disc armed with hooks of markedly unequal size; two hooks very large, one medium-sized, three small. Seminal receptacle a spiral enlargement of the oviduct. Testes do not extend to posterior end of body. A devious sperm duct between the vas deferens and penis. Penis unarmed. Vaginal pores posterior to genital

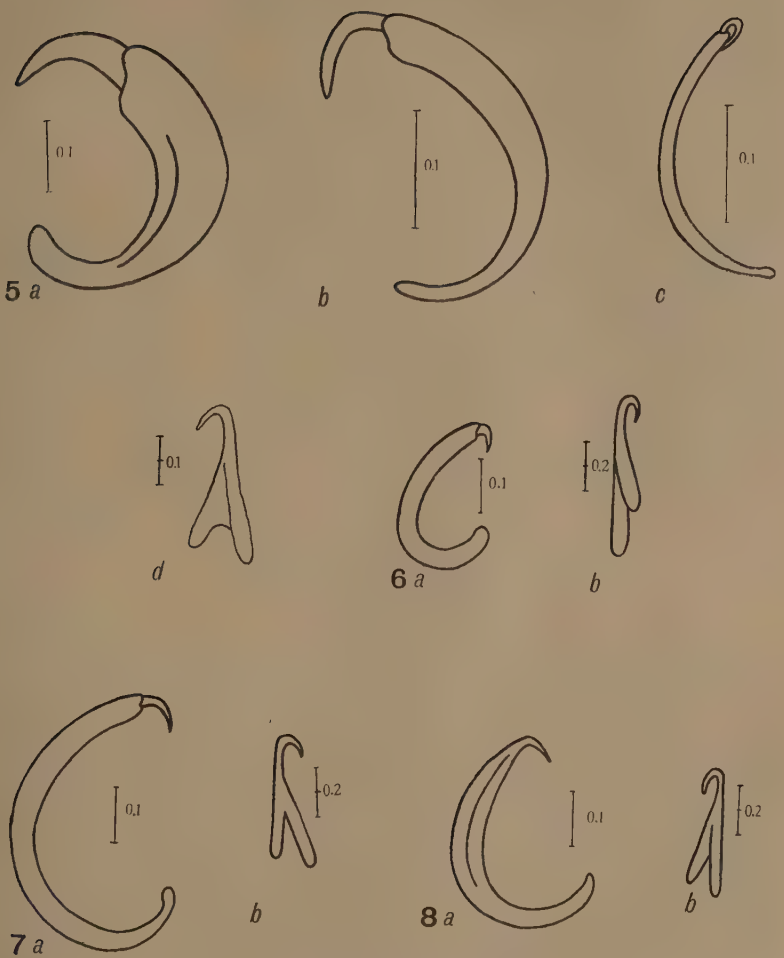

Fig. 5*a*. *Heteronchocotyle hypoprioni*, large hook.
Fig. 5*b*. *Heteronchocotyle hypoprioni*, medium hook.
Fig. 5*c*. *Heteronchocotyle hypoprioni*, small hook.
Fig. 5*d*. *Heteronchocotyle hypoprioni*, appendix hook.
Fig. 6*a*. *Squalonchocotyle ginglymostomae*, sucker hook.
Fig. 6*b*. *Squalonchocotyle ginglymostomae*, appendix hook.
Fig. 7*a*. *Squalonchocotyle microstoma*, sucker hook.
Fig. 7*b*. *Squalonchocotyle microstoma*, appendix hook.
Fig. 8*a*. *Squalonchocotyle tiburonis*, sucker hook.
Fig. 8*b*. *Squalonchocotyle tiburonis*, appendix hook.

pore. Vaginal canals paired and run parallel from vaginal pores to vitellosac. Type species—*Heteronchocotyle hypoprioni.*

This genus differs from other genera of the subfamily chiefly in the unequal size of the sucker hooks.

Heteronchocotyle hypoprioni n.g., n.sp.

Table of measurements (*in* mm.)

	Specimen				
	I	II	III	IV	V
Body length	3·6	3·1	4·5	3·4	3·6
Body width	1·2	0·9	1·5	0·94	1·2
Diameter of fixation disc	1·2	1·4	1·5	1·7	1·7
Appendix length	0·45	0·48	0·50	0·49	0·40
Appendix width	0·36	0·40	0·40	0·36	0·33
Oral sucker length	0·198	0·144	0·216	0·180	0·216
Oral sucker width	0·288	0·252	0·288	0·288	0·288
Penis length	0·216	0·198	0·270	0·170	0·170
Diameter of pharynx	0·05	0·04	—	0·05	—
Size of large hooks	0·340	0·396	0·45–0·486	0·45–0·486	0·468
Size of medium hooks	0·198	0·216	0·260	0·306	0·270
Size of small hooks	0·162–0·18	0·162–0·18	0·201–0·243	0·270	0·234–0·252
Size of appendix hooks	0·05	0·05	0·056	0·056	0·05

Note. All measurements of hooks are made through the largest diametrical dimension.

Squalonchocotyle ginglymostomae n.sp.

(Figs. 2 and 6)

Two specimens were collected from the gills of the Nurse shark (*Ginglymostoma cirratum* (Bonnaterre)) at Tortugas, Florida, in 1932.

Description. The elongate body measures 2·38 × 0·874 mm. and tapers anteriorly toward the terminal oral sucker which is slightly wider than long, measuring 0·164 × 0·21 mm. This sucker is muscular and bears minute papillae (noticeably smaller than those of *Heteronchocotyle*). The body region occupied by the oral sucker is well defined in outline. The suckers on the fixation disc are arranged in two parallel rows of three each on either side of the median line. They contain chitinous hooks of a uniform size (0·28 mm.). The hooklets of the appendix are 0·06–0·07 mm. long.

The oral sucker converges into a short prepharynx which terminates in a spherical pharynx 0·07 mm. in diameter. The intestine extends posteriorly as two highly branched crura which unite to enter the fixation disc as a common trunk. The intestine does not ramify within the fixation disc proper, but does so to a limited degree in the appendix.

The ovary, just posterior to the mid-body, is large and closely coiled upon itself. From it the oviduct proceeds in serpentine coils to the sac-like seminal receptacle lying beside the ovary. The oviduct then runs transversely to the opposite side of the intercrural space where it turns sharply forward and passes through the shell gland. Beyond the ootype it expands into a voluminous uterus. In the specimen figured (Fig. 2), the uterus contains eight to ten eggs

measuring 0·18–0·20 × 0·72 mm. Each egg is provided with two long polar filaments which intertwine in a tangled mass but do not appear continuous with those of other eggs. The uterus terminates in a genital atrium which opens ventrally through a shallow pore in the median line immediately posterior to the division of the intestine. Posterior to this opening the vaginal pores appear as two fissure-like openings on either side of the median line. The vaginae proceed posteriorly and empty separately into the vitellosac.

The testes are numerous and irregular in outline. They occupy the intercrural space posterior to the ovary. A long serpentine vas deferens leads anteriorly to the muscular penis which, in a relaxed state, lies between the vaginal pores with its opening directed posteriorly. The penis is 0·23 mm. long.

Specific diagnosis of Squalonchocotyle ginglymostomae. Length 2·38 mm.; width 0·874 mm.; hooks 0·28 mm.; appendix hooklets 0·06–0·07 mm.; intestinal crura branched, intestine ramifies in appendix but not in fixation disc; eggs 0·18–0·20 × 0·72 mm.; polar filaments long and not united in chains. Fissure-like vaginal pores slightly posterior to genital pore; muscular penis present.

This species differs from previously described *Squalonchocotyle* in that the intestine ramifies within the appendix, in the form of the vaginal pores and in its extremely small size.

Squalonchocotyle microstoma n.sp.

(Figs. 3 and 7)

One adult and one fragment (posterior end) were obtained from the gills of the hammerhead shark (*Sphyrna zygaena* (Linnaeus)) at Beaufort, North Carolina, in 1928.

Description. The specimen measures 4·29 × 1·066 mm. The anterior end tapers to a small, knob-like oral sucker, 0·3 mm. in diameter, and bears a number of small papillae. The six suckers of the fixation disc are arranged at the vertices of a regular hexagon. The chitinous hooks are uniform in size, 0·45–0·48 mm. The appendix bears a pair of hooklets 0·047 mm. long.

A short prepharynx leads from the oral sucker to the spherical pharynx (diameter 0·09 mm.). The crura of the intestine are highly branched and the common trunk ramifies extensively in the fixation disc. A single branch penetrates the appendix.

The ovary is closely coiled. The seminal receptacle is in the form of an elongate sac and lies beside the ovary. The uterus contains six to eight eggs possessing a short polar filament at each end. The body of the egg is 0·16–0·18 × 0·04–0·05 mm. The genital pore is situated medially just posterior to the pharynx. The vaginae empty separately into the vitellosac. The vitellaria are small, compact and occupy the lateral areas for the entire length of the body. A vitelline duct enters the vitellosac from either side.

The testes are numerous, irregular in outline, and extend to the posterior

extremity of the body. The vas deferens is weakly muscular and runs in a serpentine course along the median axis. The penis appears to be absent.

Specific diagnosis of Squalonchocotyle microstoma. Length 4·29 mm.; width 1·066 mm.; hooks 0·45–0·48 mm.; appendix hooklets 0·047 mm. Small oral sucker located in distinct body region. Short prepharynx. Highly branched intestine which ramifies in fixation disc. Eggs 0·16–0·18 × 0·04–0·05 mm. with short recurved filaments. Muscular vas deferens. Muscular penis absent.

This species differs from all *Squalonchocotyle* previously described in that the intestine ramifies in the fixation disc. It may be readily distinguished from *S. sphyrnae* from the same host by the small oral sucker, the absence of a wide hyaline border around the vaginal orifices, the lack of a muscular penis and the position of the appendix.

Squalonchocotyle tiburonis n.sp.

(Figs. 4 and 8)

One adult and seven young were collected from the gills of the bonnet shark (*Sphyrna tiburo* (Linnaeus)) at Tortugas, Florida, in 1932.

Description. The adult is 3·69 × 0·9 mm. and the young are 0·7–1·4 mm. long. The terminal oral sucker (diameter 0·34 mm.) is circular and of slightly greater width than that of the anterior end of the body. The suckers on the fixation disc are arranged in a circle and their chitinous hooks (0·32–0·39 mm.) are of a uniform size. The twin suckers on the appendix have a slight constriction near their base. This condition is manifest in the young as well as in the adult. The triradiate hooklets are 0·05 mm. long in both young and adult.

The oral sucker communicates directly with the pharynx (diameter 0·063 mm.). The extent of branching of the intestinal crura is indiscernible in the adult. In the young, however, the outline of the crura shows occasional small protuberances but does not exhibit distinct ramification. The intestine ramifies distinctly within the fixation disc.

The ovary is thick and much coiled. A sac-shaped seminal receptacle is present. The uterus contains a single egg bearing a short recurved polar filament at each end (Fig. 4). The body of the egg measures 0·16 × 0·05 mm. The genital atrium opens mid-ventrally posterior to the division of the intestine. The vaginal pores are in the lateral areas at the same level as the genital pore. The vitellaria are numerous and scattered and a conspicuous vitellosac is present into which the vaginae empty separately.

The testes, which are extended laterally in outline, are largely covered by the vitellaria but can be seen in the intercrural space posterior to the ovary. The remainder of the male reproductive system is obscured in the adult. The presence of a serpentine vas deferens ending in a muscular penis is indicated by the presence of these structures, in rudimentary form, in the young.

The appearance of young Onchocotylidae has not hitherto been mentioned. The specimens are similar in appearance to the adult but are much smaller,

averaging 1·4 mm. in length with the exception of one apparently younger specimen which measures only 0·7 mm. The terminal claws of the sucker hooks are less clearly defined than in the adult and the hooks themselves are smaller and more angular, being in the form of a "U". The appendix hooks, however, are the same size as those of the adult.

Specific diagnosis of Squalonchocotyle tiburonis. Length 3·69 mm.; width 0·9 mm. Hooks 0·32–0·39 mm. Appendix hooklets 0·05 mm. Suckers on fixation disc arranged in a circle. Appendix suckers with slight constriction near base. Large circular oral sucker. Intestinal crura not extensively branched; intestine ramifies within fixation disc. Egg 0·16 × 0·05 mm. with short recurved filaments.

This species resembles *S. spinacis* in constricted appendix suckers. It differs in size, host, locality and in that intestine branches in fixation disc. It differs from all other *Squalonchocotyle* except *S. microstoma* in that the intestine branches in the fixation disc. It may be distinguished from the latter by the larger oral sucker, less compact vitellaria and the shape of the testes. The lack of pronounced ramification of the intestinal crura observed in the young would serve as an additional similarity to *S. spinacis* and difference from *S. microstoma*, were this feature able to be verified in the adult.

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ON SOME REMARKABLE NEW CESTODES FROM THE AMAZON SILUROID FISH, *BRACHYPLATYSTOMA FILAMENTOSUM* (LICHTENSTEIN)

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(With Plate X, containing Figs. 1–14, and Fig. I in the Text)

The *Piraceba*, or Pirá-aïba (Piraïba) as Goeldi spells it, is a large Siluroid (my largest specimen measured 135 cm. in length) which is locally plentiful in the Amazon and has been described by Goeldi (1902). Goeldi named this species *Piratinga pirá-aïba* and it is the modern *Brachyplatystoma filamentosum* (Lichtenstein). I am indebted to Mr J. R. Norman of the British Museum (Natural History) for the modern name.

Endorchis piraeeba gen. et sp.n. and the Endorchidinae subfam.n.

In two of the half-dozen specimens of *Brachyplatystoma filamentosum* examined by me, caught in the one case about sixteen miles below Parintins and in the other about half-way between Obydos and Santarem, I discovered five examples of the very remarkable Proteocephalid tapeworm now to be described, one young wholly immature example (17 mm. long) being found in the Parintins fish and the other four in the fish caught lower down the river. My largest example of this Cestode is about 45 mm. long (the segments are all in the contracted condition, and only the last 12 mm. consist of mature segments), with a maximum breadth of 1·32 mm. (in balsam). All the segments are strongly marked with transverse creases (corresponding to distinct notches at the edges of the strobila), two to three creases appertaining to a single segment in the very immature condition (Fig. 3) and as many as twenty-two or more in fully mature segments (Fig. 2). Mature segments in the contracted condition all approximate to the square in outline (one of the largest contracted segments measures 0·99 mm. long and 0·83 mm. broad); on the other hand, all segments are extremely muscular and they can elongate so as to become over twelve times as long as broad (*e.g.* in one case the expanded mature proglottid measures 3·7 mm. long and only 0·29 mm. broad). In an elongated segment all the transverse creases disappear and the internal organs become modified in form to some extent. Mature contracted segments are less broad than the relatively immature segments immediately preceding them. Gravid segments are entirely absent in my material, no trace of an egg-containing uterus being visible. The thick cuticle and muscle sheath hinder observation of minute details in whole-mounted portions of strobila, and the

irregularly alternate cirro-vaginal openings situated very near the anterior limit of the segment are inconspicuous externally.

The scolex (Fig. 1) is relatively small and is only slightly broader than the most anterior region of the strobila, which is segmented up to the base of the scolex. In the largest worm the scolex measures 0·54 mm. broad and about 0·6 mm. long, and the segmented neck was slightly though distinctly narrower than the scolex. In the two smallest and wholly immature worms the scolex measured only 0·199 mm. broad and 0·23 mm. long (with an expanded strobila only about 10 mm. long and neck 0·08 mm. broad) and 0·36 mm. broad and 0·29 mm. long (with a contracted strobila 17 mm. long and neck 0·19 mm. broad) respectively. The scolex thus varies considerably in size according to the size of the strobila. The structure of the scolex is very characteristic. It is more or less globular with a minute "os terminale" at its apex, which is the opening of a fine duct leading into a very distinct spherical apical organ, which I believe is glandular. At the sides of the scolex, or turned forwards, are four suckers, typical save that their openings may be, or always are, triangular in form, with microscopic spines (the largest being 1·8μ long) borne upon their edges.

In a fully mature (contracted) proglottid the testes are, on a rough estimate, between 100 and 150 in number, and in contracted proglottids measure (in transverse sections) about $55 \times 36 \mu$. They lie in the medullary parenchyma in a single field, one testis deep, between the vitellaria and the anterior edge of the ovary, though the field may be regarded as divided into two by the narrow vagina (Fig. 4). The thin-walled cirrus sac, in transverse sections, measures $0{\cdot}249 \times 0{\cdot}083$ mm., and stretches over about a quarter of the breadth in a contracted proglottid. The cirrus is thick-walled, stout and unarmed, and the sac contains only two or three coils of the ductus. The vas deferens, outside the sac, forms a convoluted mass near the middle line of the segment. The vagina opens in front of the sac and extends as a fairly broad tube to the middle line of the segment and then turns back as a relatively narrow duct. The ovary consists, as usual, of two lateral masses of follicles united centrally by a transversely elongated isthmus. The remarkable feature about the ovary is that while the isthmus and large portions of the laterally placed follicle masses are medullary, the latter bear large dorsal projections which penetrate the longitudinal muscle layer and become cortical in position (Fig. 5). I have not been able to observe for certain a uterus in any of the sections, but from the presence of a distinct though narrow gap in the longitudinal muscle band ventral to the ovary I am inclined to believe that, when gravid material comes to be examined, the uterus will be found to be situated in the ventral cortex. The vitellaria are arranged in short laterally placed rows, one dorsal and one ventral on each side, in the dorsal and ventral cortex. They are individually about half the length and breadth of the testes, though their exact outlines are very difficult to observe in my transverse sections. The circular band of longitudinal muscle fibres delimiting the cortex from

the medulla is very pronounced in this species, the band (single layer) attaining a thickness (in contracted proglottids) of about 30 μ, and consisting of numerous large bundles of fibres, all closely compacted into a solid mass. I am unable to detect dorsal and ventral excretory canals and calcareous corpuscles in the parenchyma.

Systematic position

In my paper on Cestodes from Amazon fishes (1934 *a*), I described the anatomy of a new Phyllobothriid species, *Myzophorus admonticellia*. If we suppose that in this species the innermost layer of specially large muscle bundles represents the muscle layer of Proteocephalids, the testes would, in a Proteocephalid of corresponding structure, have to be regarded as medullary, while the vitellaria, the uterus and, in large part, the ovary would have to be regarded as cortical. In the above-mentioned paper I remarked that "it so happens however that in no known Proteocephalid have the organs collectively this particular arrangement relative to the muscle layer, the testes being, with the sole exception of the vitellaria, the first organs to become cortical in position, and only in the Zygobothriinae have the testes remained medullary while other organs (the vitellaria) have become cortical." It is interesting to note that *Endorchis piraeeba*, the Proteocephalid described in this paper, exactly answers to the unknown Proteocephalid discussed in the sentence above quoted. In the paper referred to I described the Amphilaphorchidinae as constituting the seventh distinct subfamily of the family Proteocephalidae, and it is evident that *Endorchis piraeeba* represents yet another and eighth subfamily, the Endorchidinae. Fig. I represents diagrammatically the various arrangements of the principal genital organs relative to the longitudinal muscle layer found in the eight subfamilies of the Proteocephalidae, and it will be seen what a very distinct subfamily the new one is, whether the uterus proves to be medullary or cortical as represented.

The definition of the subfamily Endorchidinae is: Proteocephalidae in which the testes are medullary, while the vitellaria, a large part of the ovary and (probably) the uterus are cortical. The vitellaria are dispersed and are lateral in position.

The definition of the new genus *Endorchis* is: Endorchidinae. Scolex small, bearing suckers and an apical organ. Marginal cirro-vaginal pores irregularly alternate, and the vagina opens in front of the cirrus sac. Vitellaria situated dorsally and ventrally. Type species: *Endorchis piraeeba*.

I include in Fig. I diagrams of the anatomy of an ordinary fresh-water Phyllobothriid (such as *Anthobothrium piramutāb* (Woodland, 1933*b*)) and of *Myzophorus admonticellia*, the fresh-water Phyllobothriid described in my paper (1934*a*), in order to demonstrate that it is possible that at least two Proteocephalid subfamilies (the Proteocephalinae and Endorchidinae) have been derived from Phyllobothriid ancestors by the concentration of the longitudinal muscle system and in some cases vitellaria, by the formation of suckers and

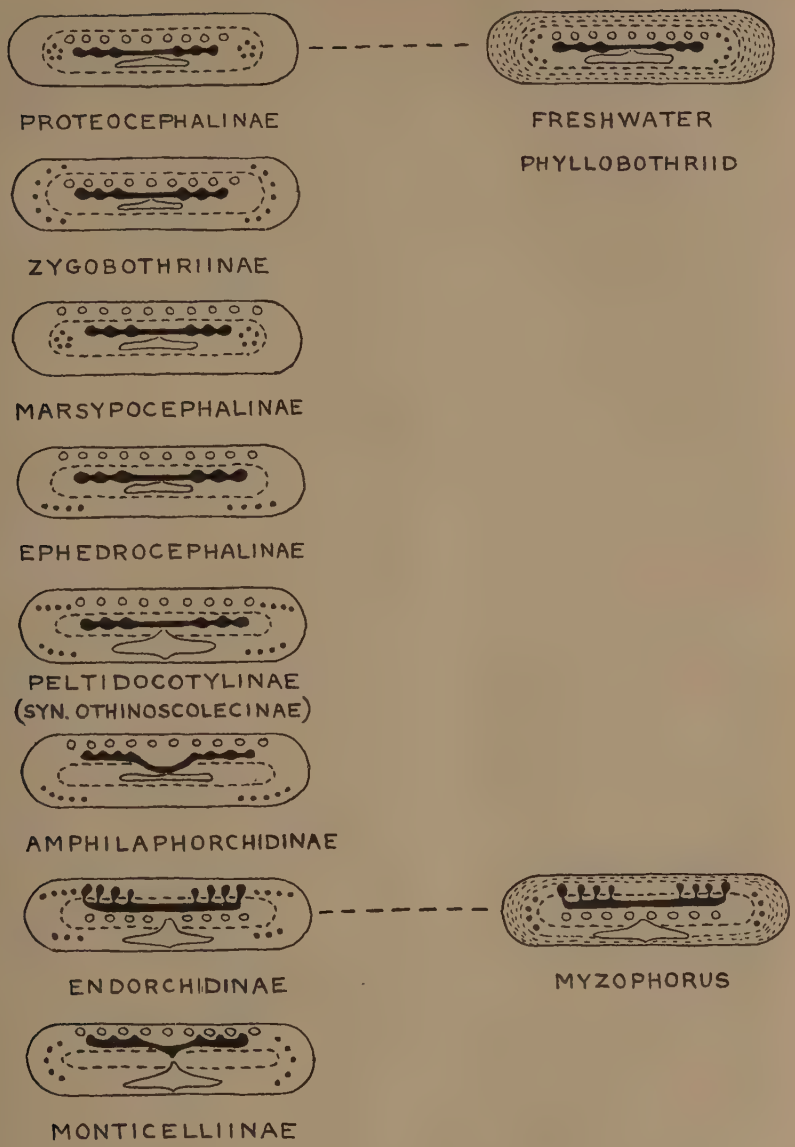
PROTEOCEPHALINAE
FRESHWATER
PHYLLOBOTHRIID
ZYGOBOTHRIINAE
MARSYPOCEPHALINAE
EPHEDROCEPHALINAE
PELTIDOCOTYLINAE
(SYN. OTHINOSCOLECINAE)
AMPHILAPHORCHIDINAE
ENDORCHIDINAE
MYZOPHORUS
MONTICELLIINAE
THE EIGHT SUBFAMILIES
OF THE PROTEOCEPHALIDAE
PHYLLOBOTHRIIDAE

Fig. I

other simple changes. I may also call to mind that even in marine Elasmobranchs Phyllobothriids have on occasion assumed Proteocephalid-like characters (unilaminate ovary, true suckers), as we see in the "*Ichthyotaenia*" *adherens* (renamed by me in 1927 *Lintoniella adherens*) of Linton (1925).

Nomimoscolex piraeeba gen. et sp.n. of the Zygobothriinae

In two *Piraeeba*, one caught about forty miles above and the other about the same distance below Obydos, I found, in the rectum of the first, four mature and gravid tapeworms of this species, and, in the anterior and middle intestine of the second, about a dozen, mostly immature, further examples. I have unfortunately made no record of the length of the largest worm, but it was probably not more than 40 mm., and the maximum breadth (of contracted mature non-gravid segments) was 0·92 mm. (in balsam). The segments are numerous and all are broader than long save some of the terminal gravid proglottids, the longest of which measured 0·9 mm. long and 0·61 mm. broad. In contracted proglottids feebly marked secondary transverse grooves and lateral notches are present but these do not mask the primary segmentation. In worms contracted anteriorly segmentation extends up to the base of the scolex and the "neck" is as broad as the scolex, but in others stretching of the anterior proglottids may produce an apparently unsegmented neck more than 2 mm. long and only 0·083 mm. broad, and all intermediate conditions exist. Though the uteri of gravid proglottids are filled with eggs, yet uterine pores have not developed in my material, but they are doubtless of the normal elongated slit type. The marginal cirro-vaginal pores are irregularly alternate and individually open a little in front of the middle point of the segment length. The vagina opens behind the cirrus sac. The scolex (Fig. 6) is, especially when compared with those of the two other known genera in this subfamily—*Zygobothrium* and *Amphoteromorphus*—both small and of normal Proteocephaline type. It consists of a central column bearing four elongated triangle-shaped (apex forwards) areas, each of which carries at its base a typical sucker, the edges of which are armed with minute spines (*ca.* 3·5 μ long). Near its apex is also borne a small though distinct bun-shaped apical organ, which is probably glandular in nature. The scolex as a whole measures 0·249–0·330 mm. in breadth and 0·18–0·23 mm. in length (base of triangular areas to apex).

The structure of a gravid proglottid (Fig. 7) is as follows. The testes lie in the medulla, and are, on a rough estimate, between 100 and 150 in number, and occupy the entire space (in surface view) between the ovary posteriorly, the border of the proglottid anteriorly, the vitellaria laterally and a median conspicuous area occupied by the large "vesicula seminalis" or mass of vas deferens coils. Towards the sides of the field the testes are in places two-deep. In transverse sections the largest testes measure $84 \times 54 \mu$. The cirrus sac measures, in transverse sections, 0·146 mm. long and 0·073 mm. broad and has a thin longitudinally striated (muscular?) wall. The contained unarmed cirrus is very thick-walled and the ductus has two or three coils. The protruded

cirrus is short and stumpy, being 0·18 mm. long and 0·099 mm. in maximum thickness. The vas deferens, as already mentioned, forms an enormous mass of coils in the centre of the proglottid and is very conspicuous both in whole mounts and in sections. Both the cirrus sac and the vagina open into an atrium. The vagina opens immediately behind the opening of the sac into the atrium and is fairly wide until it bends backward in the middle line of the proglottid ventral to the vesicula seminalis. The ovary (Figs. 7, 9) is massive and, like the testes and uterus, lies in the medulla. The vitellaria, on the other hand, lie in the cortex at the margins and form two vertical strips of follicles which are individually about half the diameter of the testes. The uterus is very large in fully gravid proglottids, occupying the greater part of the segment; it lies almost entirely in the medulla, but mid-ventrally small portions project into the cortex. The eggs (Fig. 10) are roughly spherical and thin-shelled and small, the spherical hookless embryo occupying most of the internal space, the remainder of the space being filled with yolk particles. The entire egg measures about 25·6 μ in diameter and the embryo about 14·6 μ. The usual dorsal and ventral excretory canals exist but are not always conspicuous in transverse sections. The longitudinal muscle system consists of a layer of fairly large bundles of fibres (as many as twenty may be in a bundle) well separated from each other.

This tapeworm evidently belongs to the subfamily Zygobothriinae of the Proteocephalidae, since all the principal genital organs are contained in the medullary area of parenchyma save the vitellaria (Woodland, 1933*a*). Up to the present this subfamily has only contained two genera, both named by Diesing: *Zygobothrium* and *Amphoteromorphus*. In *Zygobothrium* the large scolex has peculiar diporal suckers and is devoid of an apical organ, the cirro-vaginal pores irregularly alternate, and the vitellaria are laterally placed ventrally and dorsally extend across the entire segment. In *Amphoteromorphus* the large scolex has bilocular suckers and is devoid of an apical organ, the cirro-vaginal pores are unilateral and on the right side of the strobila, and the vitellaria are laterally placed, both dorsally and ventrally. In the present species the scolex is small and possesses suckers of the ordinary type and an apical organ is present, the cirro-vaginal pores irregularly alternate, and the vitellaria are marginal in position, consisting of two short vertically placed rows, insufficiently curved to form crescents (in transverse sections of proglottids). It is evident that the present species necessitates the creation of a third genus and I propose the name *Nomimoscolex*.

The genus *Nomimoscolex* may be defined as follows: Zygobothriinae. Scolex of small size, with normal suckers. An apical organ is present. Irregularly alternating cirro-vaginal pores. Vitellaria marginal, in two vertical rows in transverse sections. Type species: *Nomimoscolex piraeeba*.

Amphoteromorphus piraeeba sp.n. from the *Piraeeba*

In two of the half-dozen specimens of the *Piraeeba* examined by me, caught respectively in a lake near Urucará and in the main stream just below Parintins, I found the species of worm now to be described—a second distinct species of the rare genus *Amphoteromorphus* Diesing 1850.

The largest specimen devoid of scolex and end proglottid must have been 50–60 mm. in length, with a maximum breadth of 1·32 mm. (in balsam). I used this specimen and one other nearly complete specimen (*i.e.* with scolex but without end segment) measuring about 20 mm. long in part for section cutting; my other five specimens were short and immature. The proglottids are numerous and are all broader than long in my material, the longest being 0·54 mm. long and 1·09 mm. broad. The ventral uterine slit-like pores extend forwards from in front of the ovary. The marginal cirro-vaginal apertures are unilateral but with occasional irregularities, and are situated in each case a short distance behind the anterior border. The vagina sometimes opens in front of and sometimes behind the cirrus sac; in *Amphoteromorphus peniculus* I have never seen the vagina opening behind the sac. The scoleces in my material are all in the attached condition and are funnel-shaped, the edge of the end area either being expanded and thin (Fig. 12) or curled up and thick (Fig. 11). The terminal area bears, as in *Amphoteromorphus peniculus*, four bilocular suckers situated close together in the centre, and external to these are present numerous very short processes, not long and filamentous as in *Amphoteromorphus peniculus*. The breadth of the scolex (at the edge of the terminal area) in four mounted specimens is about 1·6 mm. and 1·36 mm. and the length (in two specimens) 0·58–0·66 mm. An apical organ is absent.

The structure of a gravid proglottid is as follows. The testes are numerous, about 200 in number, and when fully developed measure about $40 \times 32 \mu$ in horizontal sections. They are arranged in a single field, thin in the middle line and thicker at the edges (often two-deep—Fig. 13). The cirrus sac measures about 0·28 mm. in length and 0·10 mm. in maximum breadth and has a thin wall. The unarmed cirrus (retracted) is thick-walled and curves on itself in the sac (the distal limb being comparatively thin) and the ductus has a number of coils. The vas deferens forms a convoluted mass outside the sac as usual. The vagina opens directly to the exterior, the opening, as already stated, being sometimes in front of and sometimes behind the cirrus sac. The vagina is only very slightly dilated next the opening and the remainder of the tube shows no convolutions. The ovary is similar in form and position to that of *Amphoteromorphus peniculus*, and the uterus is also similar. The intra-uterine eggs (Fig. 14), when examined in the original formalin-glycerine preserving fluid, are somewhat similar in shape to those of *Amphoteromorphus peniculus*, but the stiff shell is more spherical and the outer covering less definite. The eggs of the present species are somewhat larger than those of *Amphoteromorphus peniculus*, the spherical shell inside the outer covering measuring about 30μ

in diameter. The hookless embryo measures about $14{\cdot}6 \times 12{\cdot}8\,\mu$. The vitellaria are of course situated in the cortical parenchyma but differ considerably from those of *Amphoteromorphus peniculus* as regards arrangement. In the latter species the vitellaria are disposed in two rows, a short dorsal and a long ventral, on each side of the segment; in the present species the vitellaria, which are about $54 \times 30\,\mu$ in transverse sections, are placed at the extreme ends of the rows of longitudinal muscle fibres and form two vertical rows—marginal—and are much less in number than in *Amphoteromorphus peniculus*. The longitudinal muscle system also differs from that of *Amphoteromorphus peniculus* in its much greater development, the numerous fibres being collected into relatively large bundles. The cortical area of parenchyma is thus clearly separated from the medullary. Small dorsal and ventral excretory canals are present but I have not observed any transverse connecting vessels or foramina secundaria, such as occur in *Amphoteromorphus peniculus*. Nor have I observed a difference of texture between the medullary and cortical areas of parenchyma.

Amphoteromorphus piraeeba differs principally from *Amphoteromorphus peniculus* in the short pimple-like condition of the adhesive processes on the terminal area of the scolex, in the more numerous testes, in the marginal arrangement of the vitellaria, and in the stronger development of the longitudinal muscle system.

The genera and species of the subfamilies of the Proteocephalidae other than the Proteocephalinae

Since I have provided a text-figure showing diagrammatically the arrangements of the four principal genital organs relative to the longitudinal muscle layer in the eight known subfamilies of the Proteocephalidae (and it appears unlikely that other arrangements will be discovered), it may prove useful to provide a complete up-to-date list of the known genera and species in these subfamilies, other than the Proteocephalinae.

Subfamily Zygobothriinae Woodland, 1933 [*a*]

Zygobothrium megacephalum Diesing, 1850.

Amphoteromorphus peniculus Diesing, 1850; *A. piraeeba* Woodland, 1934 (*vide supra*).

Nomimoscolex piraeeba Woodland, 1934 (*vide supra*).

Subfamily Marsypocephalinae Woodland, 1933 [*a*]

Marsypocephalus rectangulus Wedl, 1862; *M. heterobranchus* Woodland, 1925; *M. tanganyikae* (Fuhrmann and Baer, 1925) Woodland, 1925.

Subfamily Ephedrocephalinae Mola, 1929

Ephedrocephalus microcephalus Diesing, 1850; *E. lobosus* (Riggenbach, 1896), Mola, 1906.

Subfamily Peltidocotylinae Woodland, 1934 [*a*]
(syn. Othinoscolecinae Woodland, 1933 [*c*])

Peltidocotyle (syn. *Othinoscolex*) *rugosa* Diesing, 1850; *P. lenha* (Woodland, 1933 [*c*]); *P. myzofer* (Woodland, 1933 [*c*]).

Subfamily Amphilaphorchidinae Woodland, 1934 [*a*]

Amphilaphorchis piranabu Woodland, 1934 [*a*]; *A. myoides* Woodland, 1934 [*a*].

Spatulifer surubim Woodland, 1934 [*a*].

Subfamily Endorchidinae Woodland, 1934

Endorchis piraeeba Woodland, 1934 (*vide supra*).

Subfamily Monticelliinae Mola, 1929

Monticellia coryphicephala (Monticelli, 1891) La Rue, 1911; *M. lenha* Woodland, 1933 [*c*]; *M. megacephala* Woodland, 1934 [*b*].

Goezeella siluri Fuhrmann, 1916; *G. piramutāb* Woodland, 1933 [*b*].

I wish to express my thanks to Miss I. M. Bellis for assistance in translation from the Greek.

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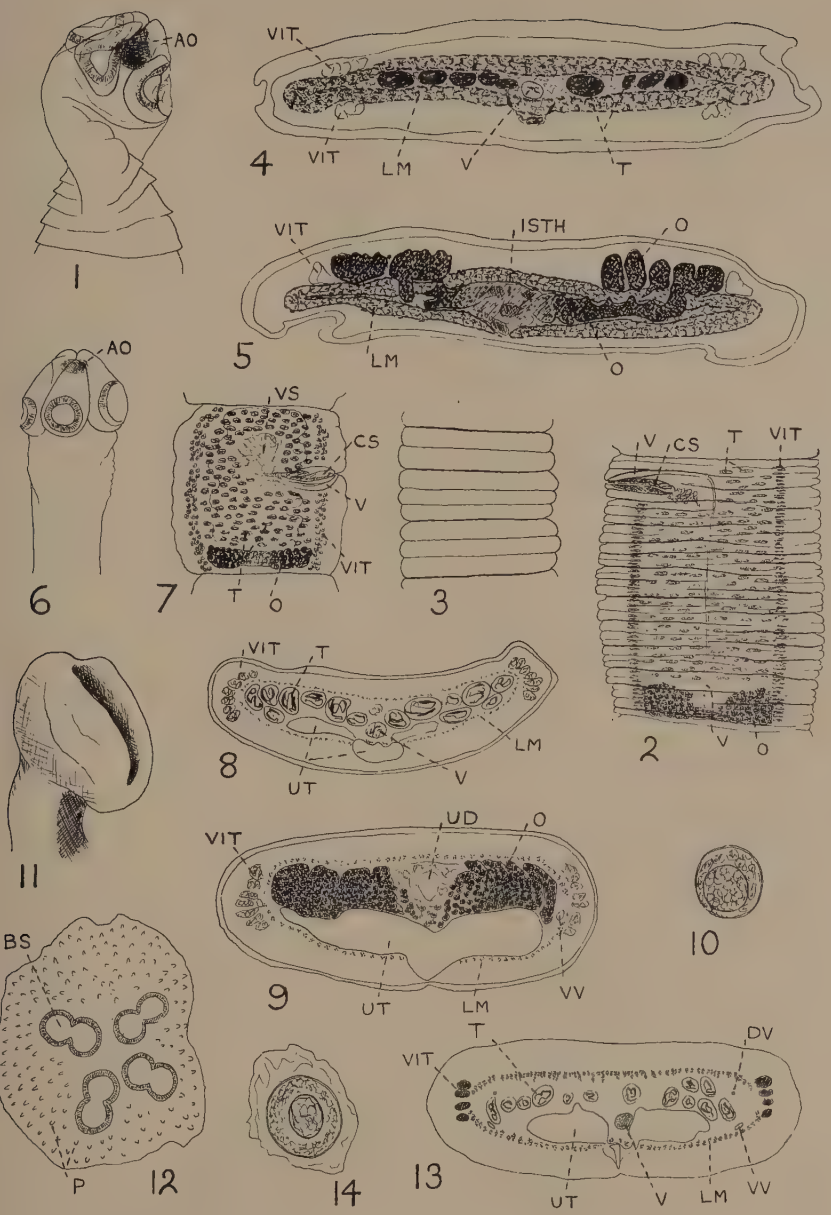
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EXPLANATION OF PLATE X

Endorchis piraeeba gen. et sp.n. (Figs. 1–5)

Fig. 1. The scolex in side view (neck twisted). ×56.
Fig. 2. A mature proglottid. ×39.
Fig. 3. Segments of immature strobila. ×39.
Fig. 4. Transverse section of mature proglottid anterior to the ovary. ×87.
Fig. 5. Transverse section of mature proglottid in region of ovary. ×87.

Nomimoscolex piraeeba gen. et sp.n. (Figs. 6–10)

Fig. 6. The scolex in side view. ×56.
Fig. 7. Gravid proglottid (only the outlines of the uterus are shown and the contained eggs have been omitted in the drawing). ×39.
Fig. 8. Transverse section through gravid proglottid anterior to ovary. ×56.
Fig. 9. Transverse section through gravid proglottid in region of ovary. ×87.
Fig. 10. Intra-uterine egg sketched in formalin. ×395.

Amphoteromorphus piraeeba sp.n. (Figs. 11–14)

Fig. 11. The scolex in side view, with the edges of the terminal disc contracted. ×27.
Fig. 12. End-on view of the surface of the terminal disc showing bilocular suckers and small processes. ×27.
Fig. 13. Transverse section of gravid proglottid anterior to the ovary. ×39.
Fig. 14. Intra-uterine egg sketched in formalin. ×395.

Lettering of figures: *AO*, apical organ; *BS*, bilocular sucker; *CS*, cirrus sac; *DV*, dorsal excretory canal; *ISTH*, ovarian isthmus; *LM*, longitudinal muscles; *O*, ovary; *P*, short processes on upper surface of scolex; *T*, testes; *UD*, uterine duct; *UT*, uterus; *V*, vagina; *VIT*, vitellaria; *VS*, coils of vas deferens; *VV*, ventral excretory canal.

(*MS. received for publication* 31. I. 1934.—Ed.)

NEMATOSTRIGEA SERPENS (NOM.NOV.) A REDESCRIPTION OF *HOLOSTOMUM SERPENS* NITZSCH 1819

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(With Plate XI)

The material upon which this paper is based was secured in May, 1930, from a specimen of *Scopus umbretta bannermani* C. H. B. Grant (Ciconiiformes) which had been shot by a native on the Buzi River in Mozambique, about 40 miles from the eastern border of the Melsetter District of Southern Rhodesia. Several hours elapsed before the bird was brought into camp and its skin prepared for museum purposes, and our parasitological examination of the specimen was carried out under some difficulty with failing light. No worms were found in the alimentary tract, but on removing the liver and teasing this organ apart about twenty trematodes were recovered. To the naked eye these appeared very similar to schistosomes and, accepting this as a tentative diagnosis, we assumed that the parasites had been loosened from an abode in the branches of the hepatic portal vein. Considerably later, when we sought to identify the formalin-preserved specimens, it became evident that we had chanced upon an infection with a species of "holostome" closely related if not identical with that atypical and obscurely known form, *Holostomum serpens*.

Like all other members of the Strigeidae (=Holostomidae *in parte*), *H. serpens* has been found previously only in the intestine. On this account we are now inclined to suspect that our finding of the worms in the liver may have been due to their migration from the duodenum into the bile duct, perhaps after the death of the host. However, this hypothesis is not easy to conceive, and future observations may show that the development of an extraordinarily slender body in *Holostomum serpens* is an adaptation towards living in the situation now reported.

This is the first report of the parasite outside Europe. It does not appear to exhibit any narrow limitations as to host specificity. It was first discovered in *Pandion* (=*Falco*) *haliaëtus*, the fish-hawk or osprey (Falconiformes), by Nitzsch in 1819; its next record, according to the comprehensive account of Szidat (1929), was based on a specimen found in a gull, *Larus marinus* (Charadriiformes), by Mühling in 1898.

Despite the antiquity of *Holostomum serpens* in helminthological literature, no description of its morphology, compatible with modern requirements for establishing its systematic status, has as yet been furnished. In his preliminary account of the review of the genus *Strigea* Abildgaard 1793, Szidat (1928),

referring to *Holostomum serpens*, remarked: "wahrscheinlich für diese Art auch eine neue, selbständige Gattung gebildet werden muss." In the following year we were provided by Szidat with a brief account and a useful sketch of the main features of the parasite based on a specimen collected by Lühe from the type host and deposited in the Königsberg Museum. In this work, Szidat deals with *H. serpens* as a species of uncertain generic affinity for which he still retains the old, and now inadmissible, name, *Holostomum*.

While we entertained no doubt as to our African material being congeneric with *H. serpens*, in certain details it does not coincide with Szidat's description of the species. However, Dr Szidat, who was kind enough to give me his opinion on some specimens which I had sent, assures me that it is "without doubt identical with *Holostomum serpens* Nitzsch." The species must therefore be recognised as capable of assuming a wide range of forms, examples of which we have illustrated in Figs. 1 and 2.

In redescribing the species, it becomes necessary to refer to it by an appropriate name and, as suggested by Szidat (*loc. cit.*), we find it necessary to establish a new genus, since it cannot be accommodated in *Strigea*, or in any of the several new genera that have been erected to take care of the heterogeneous assemblage of species that formerly paraded under the title *Holostomum*.

The name *Nematostrigea* is accordingly proposed with the following generic diagnosis:

Strigeidae, cylindrical and much elongated in form. Relatively minute fore-body of conical shape. Holdfast organ well developed, composed of two protrusile, foliaceous lobes. Hind-body consists of long, narrow, highly extensile "neck" region and a short terminal part to which the genitalia are confined. Genital cone powerfully developed and eversible through the spacious copulatory bursa. Clusters of vitelline follicles arranged in strands which extend from behind holdfast gland to ovary, from which point isolated follicles, disposed predominantly in the ventral field, penetrate to the vicinity of the genital cone.

Type species: *Nematostrigea serpens* (Nitzsch).

Synonyms: *Holostomum serpens* Nitzsch 1819; *Amphistoma serpens* Rudolphi 1819.

Description[1]. Preserved specimens have the appearance of irregularly twisted fragments of slate grey thread. At one end, the fore-body stands out as a slight thickening or knot; for two to three millimetres, the opposite extremity is substantially thickened with the rim of the bursa flaring outwards like a bell. The length of the body varies considerably according to the state of contraction or relaxation of the numerous bundles of longitudinal muscles concentrated especially in the so-called neck region of the hind-body. Contracted specimens may measure not more than 8 mm. in total length, while relaxed specimens can

[1] The writer records with appreciation his indebtedness to Dr George R. Larue of the University of Michigan for aid in elucidating the morphology of the material here described.

attain a length of more than 20 mm. with the neck region reduced in thickness to that of a very fine filament. It is upon the more stumpy, contracted specimens that the following description is mainly based. In such specimens the fore-body is about 0·7 mm. long and about 0·25 mm. at its broadest point; neck region has a fairly uniform width of about 0·2 mm.; the terminal part of hind-body is about 0·4 mm. wide.

Cuticle smooth but thrown into pleats in the neck region which then assumes a coarsely striated appearance.

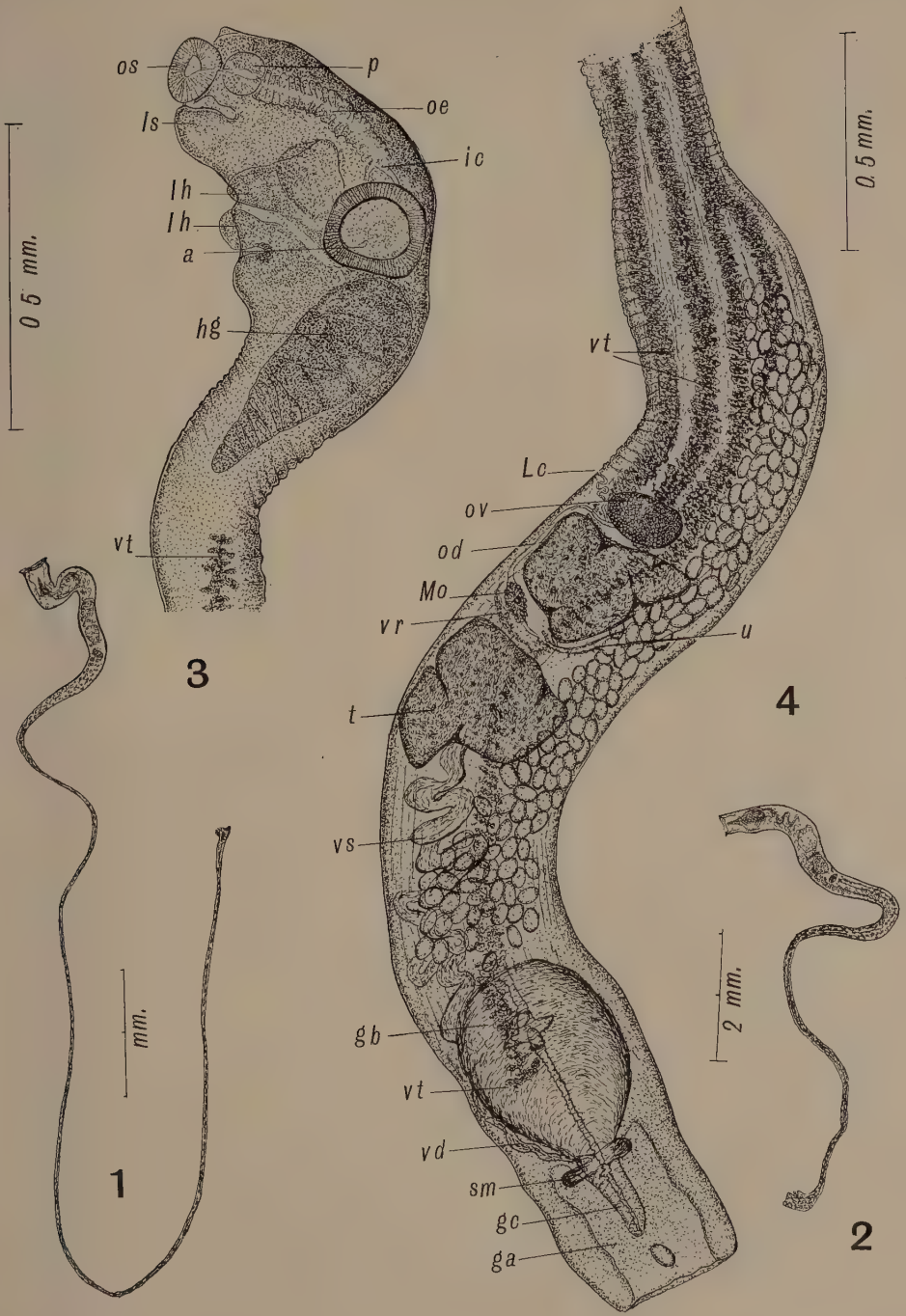
Fore-body (Fig. 3). Highly variable in shape but often comparable with an elongate cornucopia, from the opening of which the two foliaceous lips of the holdfast organ may prominently project. Oral sucker terminal, 0·10–0·12 mm. in diameter, flanked on either side by labile sucking cups or auricles. Pharynx round or ellipsoidal according to angle of vision, about the same size as oral sucker or only slightly smaller. Oesophagus, surrounded by unicellular glands, of appreciable length in the well-expanded specimen, bifurcates just anterior to the powerful acetabulum which has a diameter of 0·18 mm. Intestinal caeca are found in serial sections to extend down to the region of genital cone. Large adhesive or holdfast gland composed of lobules of deeply staining granular cells, situated at base of fore-body.

Excretory system. Two principal vessels, a large one lying dorsally and a narrower one ventrally, running the full length of the neck region. These vessels are confluent at a point near the base of the holdfast gland, whence smaller vessels ramify in the parenchyma of the fore-body. Distribution of excretory system or the location of the excretory pore could not be followed in the hind-body.

Genitalia. Vitellaria are profusely developed in the neck region. Compact follicles are arranged in a series of discrete columns in the peripheral parenchyma, between the bundles of longitudinal muscle fibres. The columns tend to lose their individuality and coalesce in the region of the ovary. In the wider terminal part of the hind-body the vitellaria suffer reduction but a scattered band of follicles, confined mainly to the ventral field, extend down to the region of the genital cone. Transverse collecting duct, swollen into a prominent vitelline reservoir, is situated between the testes.

Testes, slightly lobed, approximately equal in size (*ca.* 0·35 mm. in width) arranged in tandem in the middle third of the widened portion of hind-body. Vasa efferentia emerge on posterior border of each testis, proceed anteriad to unite in the region of the ovary, whence the vas deferens runs posteriorly. Behind the posterior testis, the vas deferens is dilated to form a conspicuous seminal vesicle which, as a muscular ejaculatory duct, then proceeds down the dorsal side of the genital bulb, pierces the thick wall of this organ and opens in the vicinity of the sphincter band.

Ovary ellipsoidal, measuring about 0·12 by 0·18 mm., located in median field, almost contiguous with anterior border of anterior testis. Oviduct arises latero-posteriorly, soon gives off the long, coiled Laurer's canal and, bending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sharply, continues down the dorsal face of the anterior testis. In the intertesticular space the oviduct receives the duct from the vitelline reservoir and from the well-developed Mehlis gland. The fertilised ova pass into the uterus which proceeds anteriad to the point where the hind-body narrows to form the neck region and then doubles back on itself posteriad. The eggs, of which it is estimated considerably more than 100 may be stored in the uterus, are expelled through a powerful ovejector apparatus. This organ, having a pyriform shape, may be described as being composed of two parts: a *spherical bulb*, with thick walls composed of muscles arranged in criss-cross fashion and tapering off into a *genital cone* which presumably serves as an intromittent organ in copulation, for it may be everted through the genital atrium. At the junction between the bulb and cone is a strong annular sphincter which arises from the walls of the surrounding genital atrium or bursa. The uterus enters the bulb anteriorly and the eggs pass through the narrow but highly corrugated lumen of this organ.

Eggs, with thick, golden yellow shell, measure from 90 to 100 microns in length by 52 to 56 microns in maximum width.

COMMENT ON SYSTEMATIC STATUS

On account of the restriction of the vitellaria to the hind-body, a character which has been invested with primary value in the classification of the Strigeidae, the genus *Nematostrigea* is to be associated with such genera as *Apatemon* Szidat, *Cotylurus* Szidat, *Cardiocephalus* Szidat and the more recently proposed *Pseudostrigea* Yamaguti 1933. Of these, it appears to bear greatest resemblance to *Cardiocephalus* with its well-developed neck region, but the difference in the relative size and structure of the fore-body, measured by the standards now in vogue in the classification of the various families of Strigeioidea, warrants our making the generic distinction.

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EXPLANATION OF PLATE XI

Nematostrigea serpens

Figs. 1 and 2. Two complete specimens to show extensibility of "neck" region.
Fig. 3. Fore-body. Lateral view; optical section.
Fig. 4. Hind-body. Lateral view; optical section. Composite figure showing disposition of genital organs.

Abbreviations: *a*, acetabulum; *ga*, genital atrium; *gb*, genital bulb; *gc*, genital cone; *hg*, holdfast gland; *ic*, intestinal crura; *Lc*, Laurer's canal; *lh*, lip of holdfast organ; *ls*, labile sucking cups; *Mo*, Mehlis gland and oötype; *od*, oviduct; *oe*, oesophagus; *os*, oral sucker; *ov*, ovary; *p*, pharynx; *sm*, sphincter muscle; *t*, posterior testis; *u*, ascending limb of uterus; *vd*, vas deferens; *vr*, vitelline reservoir; *vs*, vesicula seminalis; *vt*, vitellaria.

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IXODES RICINUS IN RELATION TO ITS PHYSICAL ENVIRONMENT: THE INFLUENCE OF CLIMATE ON DEVELOPMENT

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(With 6 Figures in the Text)

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I. Introduction

During the last few decades, a considerable amount of experimental work has been carried out on the relations of insects to their environment: in certain groups such studies have advanced to an extent which renders possible tentative efforts at accurate prediction of their activities and spread. Among the arachnids, however, the experimental study of ecological relationships has been almost entirely neglected, and this despite the fact that several species, especially among the mites and ticks, are of marked economic significance. Since 1892–3, when the researches of Smith and Kilborne on piroplasmosis in cattle were published, ticks have been proved to be vectors of numerous important diseases of man and animals in many parts of the world, and much

knowledge of their bionomics has been gained through observations conducted in the field and in the laboratory. The ecology of ticks under precisely controlled conditions, however, provides a practically unexplored field.

The only tick of major importance in Britain is *Ixodes ricinus*, which is the vector of redwater in cattle, and of louping-ill and tick-borne fever in sheep. In 1929 an investigation of its bionomics was begun by the writer, and, following its incrimination as the carrier of louping-ill and tick-borne fever, a comprehensive study of its general environmental relationships was undertaken. An account of certain field observations on its distribution, seasonal activities and host relationships has already been published (MacLeod, 1932).

The present paper describes laboratory experiments upon the effects and intereffects of different physical factors in the environmental complex on the development of gorged *Ixodes ricinus*, hereafter referred to merely as the tick or ticks.

II. Influence of temperature and humidity on development

(*a*) *Methods*

The ticks used in the experiments described below were bred for one or more generations in the laboratory. The methods of breeding have already been described (MacLeod, 1932).

For experiments at constant temperatures of 22·5° C. and over, ordinary gas-heated thermostats set at different temperatures were used. The temperature of each thermostat did not vary more than plus or minus half-a-degree Centigrade. There is a slight variation of temperature between different parts of thermostats of this type, but the possible error from this source may be obviated to a certain extent by keeping the tick containers as far as possible in the same position in the incubator for all experiments. A temperature of 20° C. was obtained by a water-bath with a gas-control mechanism, with the addition of the usual cold-water circulation for thermostats at 14–16° C. A portable electric incubator, kept in an outhouse in winter, provided a steady temperature of 10° C., while the cold store was used for the lowest temperature of 2–3° C. Constant humidities were obtained by using solutions of caustic potash, the relative humidity over which remains practically constant for given concentrations throughout the biological range of temperature (Paranjpe, 1918). Pure caustic potash sticks were used for making the solutions, and their anhydrous purity tested before use by titration of a given solution against standard hydrochloric acid. The ticks for each experiment were placed in a test-tube, the mouth of which was covered by muslin, held taut by a rubber band. Each tube was placed in a milk sample bottle containing some caustic potash solution of the requisite concentration, and the bottle tightly closed with a rubber stopper. After exposure of the system for some hours to the appropriate temperature, the stopper was released and replaced; thus a normal atmospheric pressure in the closed system was obtained. When the ticks approached the moulting stage the tubes were examined daily and the

number of moults recorded. In some of the experiments, where the ticks were left for 2 or more months over KOH solutions, there was a tendency for K_2CO_3 to form, through absorption of CO_2. This, however, would not appreciably affect the result since, according to Paranjpe, 2 g. K_2CO_3 equals 1·5 g. KOH, as far as vapour pressure is concerned.

(*b*) *Experimental data*

Two series of experiments with larvae, and three with nymphs, were carried out at different combinations of temperature and humidity. Four to eight gorged nymphs and ten gorged larvae were used in each experiment; the results recorded below have, therefore, been obtained from approximately twenty larvae and eighteen nymphs at each combination. The ticks in each series were submitted to experiment on the same day on which they had completed engorgement. The shortest, longest and average time (in days) from engorgement to moulting, and the percentage mortality are given in tabular form (Tables I and II).

Table I. *Larvae. The number of days taken for development, and the mortality at different temperatures and humidities.*

Relative humidity		Temperature °C. 22·5	26	27·5	30	32·5	35
100	Shortest	48	25	22	19	17	18
	Longest	64	37	34	25	22	28
	Average	56	31	28	23	20	21
	Mortality (%)	0	5	0	0	0	35
95	Shortest	34	28	24	22	19	22
	Longest	70	48	37	28	28	30
	Average	55	38	30	25	24	28
	Mortality (%)	15	15	20	20	10	90
90	Shortest	40	31	28	23	28	—
	Longest	70	54	37	32	31	—
	Average	58	41	32	26	29	—
	Mortality (%)	30	15	5	15	65	100
85	Shortest	51	40	34	23	—	—
	Longest	64	61	41	32	—	—
	Average	59	48	37	28	—	—
	Mortality (%)	55	50	80	85	100	—
80	Shortest	—	—	—	—	—	—
	Longest	—	—	—	—	—	—
	Average	—	—	—	—	—	—
	Mortality (%)	100	100	100	100	—	—

The following points in the tables are worthy of remark:

(1) There is a marked individual variation in the time taken for development; ticks of identical history, kept under similar conditions in the same tube, exhibited variations of as much as one-quarter or even one-third of the longest time recorded for these particular conditions.

(2) The lower limiting conditions of humidity for survival and development of gorged ticks is surprisingly high, being in the neighbourhood of 80 per cent. saturation at the lower medial temperatures, and 90–95 per cent. at the upper

temperatures. The larval stage appears to be even more intolerant of aridity than the nymphal stage, the lower limit at 22·5° C. being about 85 per cent.

(3) Under the same conditions of temperature and humidity, the gorged nymph required longer time for development than the gorged larva.

(4) There appears to be a higher mortality among nymphs than among larvae.

Table II. *Nymphs. The number of days taken for development, and the mortality at different temperatures and humidities.*

Relative humidity		Temperature °C. 22·5	25	27·5	30	32·5	35
100	Shortest	68	47	35	25	23	23
	Longest	86	74	47	33	31	30
	Average	78	61	41	30	28	28
	Mortality (%)	33	26	7	20	14	38
95	Shortest	—	46	31	27	28	30
	Longest	—	72	49	28	41	—
	Average	—	60	39	30	33	—
	Mortality (%)	—	0	0	30	13	50
90	Shortest	50	44	31	27	31	36
	Longest	59	72	47	36	40	—
	Average	55	49	37	31	38	—
	Mortality (%)	30	14	0	0	29	80
85	Shortest	72	55	40	32	31	—
	Longest	82	74	45	44	—	—
	Average	79	65	44	37	—	—
	Mortality (%)	30	0	43	0	50	100
80	Shortest	59?	—	39	34	—	—
	Longest	—	—	63	40	—	—
	Average	—	—	49	37	—	—
	Mortality (%)	88	100	50	63	100	—

(*c*) *Development at constant temperatures and humidities*

If the time taken for development of gorged larvae in saturated air is plotted against temperature, within the limits given in Table I, it will be seen that the curve is of the nature of a hyperbola (Fig. 1). In this respect it agrees with the general curve for insect development at medial temperatures.

The work of Pearls (1914), Bodenheimer (1928) and others has shown that the correlation curve between temperature and development of various insects is a true equilateral hyperbola for the medial temperature range, the product of the effective temperature and the time being a constant. The curve for tick development (Fig. 1) suggests that the law of thermal constants holds good for development of ticks also, within the medial temperature range. The application of the thermal-constant theory to the development curve will be discussed in detail later.

An examination of the data in Table I reveals the fact that the temperature at which development takes place most rapidly is not a fixed value irrespective of the humidity conditions, but varies with different humidities. If the reciprocal values of the time taken for development are plotted against temperature, the resulting curve represents the relative velocity of development

at different temperatures. In Fig. 2 the velocity-temperature curves of larvae at 100, 95 and 90 per cent. saturation have been drawn. The curves are based on the shortest times for development (Table I). The curves illustrate three points of interest.

(1) Saturated air affords the optimum moisture conditions for development at temperatures over 26° C., the velocity for any temperature over this decreasing with decreasing humidities. The greatest developmental velocity obtained was in saturated air.

(2) Under optimum humidity conditions, 32·5° C. appears to represent the temperature at which development proceeds most rapidly.

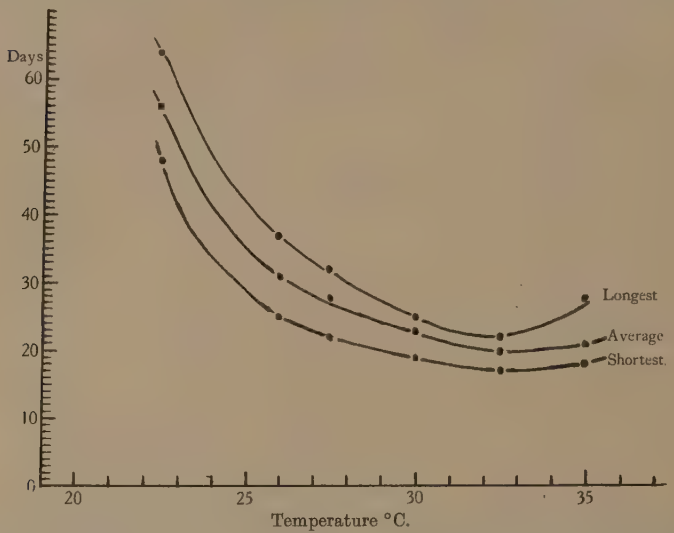

Fig. 1. Development curves of gorged larvae at different temperatures. Shortest, longest and average times for development (Table I).

(3) With decreasing humidities the point at which the greatest developmental velocity is exhibited tends to move down the temperature scale; for 100 per cent. saturated air the difference between the velocity at 32·5° C. and that at 35° C. is insignificant; for 95 per cent. saturated air the velocity is definitely greater at 32·5° C., while in air, 90 per cent. saturated, 30° C. is the optimum temperature as regards velocity.

A study of Table II shows that the same general principles hold for development of gorged nymphs, except that the velocity curve remains stationary from 32·5° to 35° C., instead of dropping slightly, as in the case of larvae.

It has been shown that the optimum temperature for developmental velocity is dependent on humidity. The converse of this also holds—the optimum humidity for development is dependent on temperature. This is

more clearly illustrated in the case of the gorged nymphs. Fig. 3, giving the velocity-humidity curves of gorged nymphs at different temperatures, shows that with decreasing temperatures the point at which developmental velocity is greatest tends to move down the humidity scale; at 30° C. the optimum humidity for developmental velocity is 100 per cent. saturation, at 27·5° C. it is between 90 and 95 per cent., and at 25° C. it is 90 per cent.

The same principle operates in the case of larvae, the deviation of the optimum humidity from saturation beginning at temperatures below 26° C.

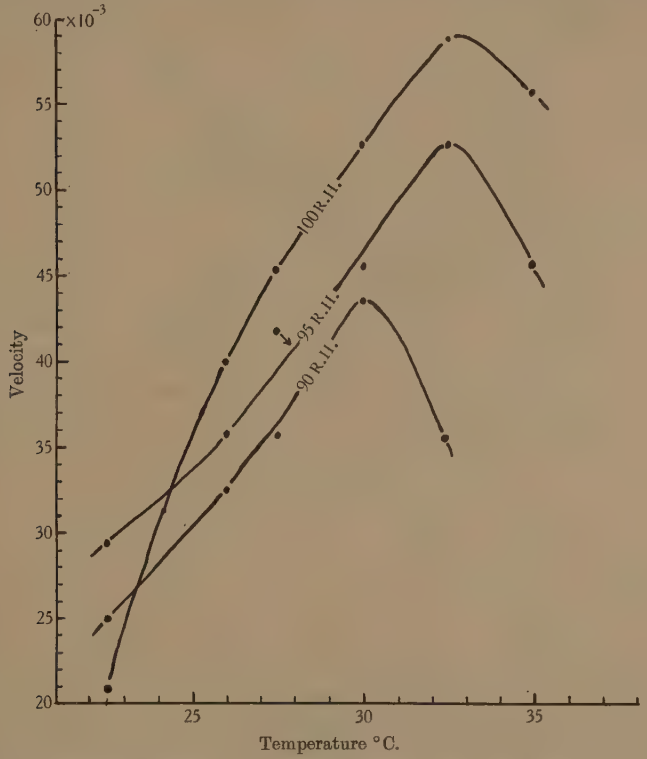

Fig. 2. The velocity-temperature curves of larvae at different humidities. The curves are based on the highest velocities obtained (Table I).

(*d*) *Development at fluctuating temperatures*

The recorded data on the influence of fluctuating temperatures on the development of insects is conflicting. Uvarov (1931) summarises the results of various workers, some of whom obtained a retardation, and others an acceleration effect by subjecting eggs and larvae of different insects to fluctuating temperatures. More recently, MacLagan (1932) found that violently fluctuating temperatures produced an initial acceleration in the rate

of growth of *Smynthurus* nymphs, but eventually proved harmful, resulting in a rapid decrease in rate.

Other workers have studied the effect on the development of various insects of preliminary exposures to high or low temperature, but this does not properly belong to the subject of fluctuating temperatures, and will be discussed in relation to the tick in the next section. The effect of temperature

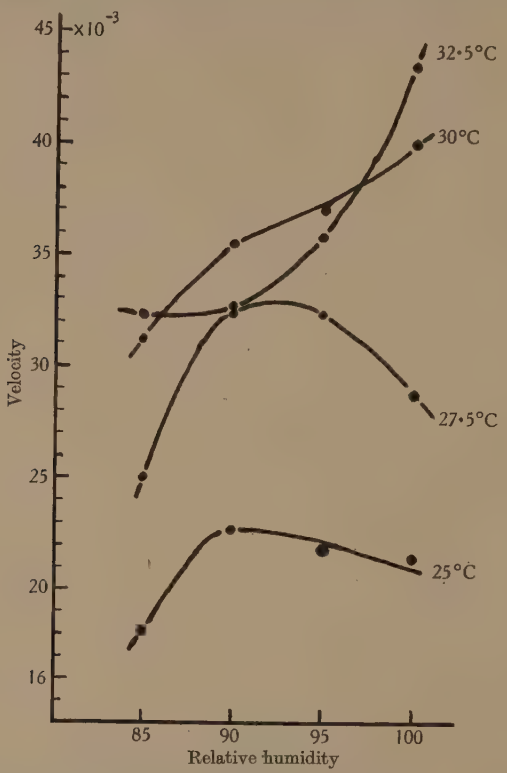

Fig. 3. The velocity-humidity curves of nymphs at different temperatures. The curves are based on the highest velocities obtained (Table II).

fluctuations as these might occur in nature on the physiological response to particular temperatures is important, and must first be investigated before a true conception can be formed of the influence of temperature as a climatic factor, since organisms in nature are not subjected to the constant temperature conditions obtaining in laboratory experimentation.

Gorged larvae which had been kept at room temperature for a few days were subjected to different temperatures as follows. Two control tubes, with fifteen larvae in each, were exposed to 25° and 35° C. respectively. A third tube, containing thirty larvae, was alternated every 3 days between 25° and 35° C.

To avoid the condensation of water on the bodies of the ticks, inevitable in saturated air when a tube is brought from 35° to 25° C., the experiment was carried out with a uniform R.H. of 95 in all the systems.

The results of the experiment may be summarised as follows:

The controls at 35° C. moulted from the 27th to the 30th day; those at 25° C. moulted from the 33rd day onwards; those exposed to fluctuating temperature moulted from the 33rd day onwards.

The experiment was repeated in saturated air, with narrower limits of fluctuation. Fifteen ticks were exposed to limits of 30° and 35° C., the temperature being alternated every 3 days. Control tubes of 10 larvae each were exposed to constant temperature conditions at these limits.

Moulting occurred as follows:

	Shortest	Average
Controls at 35° C.	19	22
Controls at 30° C.	20	25
Alternating tube	23	26

Violent fluctuations to extreme limits were then tested. A tube of twenty ticks was alternated every third day between 2° and 30° C. By the eighth alternation of temperature all the ticks were dead, probably as a result of the abrupt and violent changes of temperature.

It follows that, for the tick, moderate fluctuations of temperature within the favourable limits do not result in an appreciable acceleration of development; the results suggest that, if anything, a slight retardation effect is produced. The effect on development of maintaining the tick for periods of days at a succession of static temperatures is discussed in Section IV.

III. The temperature-velocity curve at fixed temperatures

(*a*) *The possibility of stimulation by low temperatures*

Were the curve of development of the tick a true equilateral hyperbola, the reciprocal curve, expressing the velocity of development, would therefore be a straight line. It is obvious, however, that the curve of development throughout the full biological range of temperature cannot be a true hyperbola, since the tick will not survive indefinitely at low temperatures, and retardation of development and death occur at the upper limit. Prochnow (cited by Uvarov, 1931) traced the developmental velocity curve of various insects throughout the full temperature range, and found that above and below the medial temperature range the straight-line character of the curve was departed from. The method he adopted was to expose the insects for a certain interval at a low temperature, then to transfer them to a favourable temperature, and allow them to complete their development. The fraction of their development completed at the low temperature in the given time was reflected in the differences between the time required to complete development at the favourable temperature and the time required for total development at this temperature. From this the rate of development at the low temperature was calculated. The work of Bodine and Parker on the development of grasshopper

eggs (Uvarov, 1931) suggests a possible source of error in this method, however. These workers demonstrated that with grasshopper eggs a preliminary exposure to low temperature stimulated the eggs to a more rapid development at favourable temperature than would normally occur. The possibility, therefore, had to be considered that, if Prochnow's method were used to determine the velocity of development at low temperatures, results indicating an apparent development at the lower temperature might in reality be due to an acceleration of the normal rate at the higher temperature, resulting from the stimulating effect of the preliminary exposure. To test this possibility, the following experiment was set up.

Larvae were exposed to temperatures of 2–3° C. and 9–10° C. for a period of 60 days, then transferred to 27·5° C., R.H. 95. It was reasoned that, if a genuine stimulation resulting in accelerated development had occurred, this would be at least as marked, if not more so, in the ticks exposed to 2–3° C. as in those exposed to 10° C.

Moulting occurred in both lots between the 31st and 37th day after removal to 27·5° C. The average time for complete development at 27·5° C., 95 R.H., is 30 days; it would appear, therefore, that, in so far as the tick is concerned, stimulation by exposure to low temperature does not produce an acceleration of developmental velocity at subsequent temperatures, but possibly a slight retardation.

It is, therefore, permissible, if the possibility of a slight degree of retardation occurring even at moderately low temperatures be disregarded, to use this method in the case of ticks to determine the velocity of development at temperatures below the medial temperature range.

(*b*) *Development at* 20° C.

An experiment was first set up in which gorged larvae were exposed at different humidities to a temperature of 20–21° C., and left until moulting commenced. One larva at R.H. 100 moulted on the 93rd day; at 90 R.H. two moults occurred on the 88th day, and at 85 R.H. two occurred by the 120th day. No further moults occurred.

The experiment was repeated, six nymphs and ten larvae being placed at 20° C. at each of the following relative humidities: 100, 95, 90, 85, 80 and 75.

The results were as follows:

Table III

	Larvae		Nymphs	
Humidity	No. of moults	Time in days	No. of moults	Time in days
100	—	—	—	—
95	—	—	—	—
90	4	160–180	1 1	110 170
85	—	—	2 2	83–90 170
80	—	—	—	—
75	—	—	—	—

No further moulting had occurred by the 190th day. Practically all the remaining larvae were found to be dead. The surviving unmoulted nymphs were transferred on the 190th day to 27·5° C., 100 R.H.

Moulting occurred as follows:

Table IV. *Nymphs.*

R.H. when at 20°	100	95	90	80	75
No. of days at 27·5° C., 100 R.H.	4	7	4	10	13
	7	—	10	13	—
	10	—	10	—	—
	10	—	—	—	—
	10	—	—	—	—

Since the average time taken for complete development at 27·5° C., R.H. 100, is 41 days, the amount of development which took place in from 4 to 10 days represented approximately from 1/4 to 1/10 of the total. That is, in 190 days, the unmoulted nymphs at R.H.s of 100 and 90 had completed from 3/4 to 9/10 of their development, and, therefore, would theoretically require from about 20 to 60 more days. The shortest and longest periods for nymphs at 20° C., worked out from these results, are as follows:

R.H.	100	90	85	80	75
Shortest	210	110	83–90	250	280
Longest	250	250	170	280	—

Thus we find that at 20° C. the humidity at which development is most rapid is 85 per cent., the velocity decreasing at humidities above and below this point.

The developmental velocity of gorged larvae at this temperature was found as follows:

Tubes each containing ten larvae were set at 20° C., 100, 90 and 80 R.H. After exposure for 68 days they were removed. Those at 80 R.H. were found to be dead. The remaining two tubes were set at 27·5° C., 100 per cent. saturation. Moulting occurred as follows:

Table V. *Larvae.*

100 R.H.		90 R.H.	
No. of moults	Days at 27·5° C.	No. of moults	Days at 27·5° C.
1	7	2	7
3	10	1	10
3	13	1	17
3	17	Remainder died	

The time required by larvae at 27·5° C., 100 per cent., averages 28 days; that is, 7/28–17/28 of the total development was completed at 27·5° C. and, therefore, 68 days represents from 11/28 to 21/28 of the total number required at 20° C. This makes the time at 20° C. required by gorged larvae for complete development approximately 90–170 days. Combining this result with the

result of the first experiment, we find the time required for development of gorged larvae at 20° C. to be as follows:

R.H.	100	90	85
Days	90–170	88–170	120–?

In these results, as in the results given in Tables I and II, there is a clearer indication in the case of the nymph than in that of the larva of a deviation of the optimum humidity from saturation with decreasing temperatures.

(c) *Development at temperatures of* 15° C. *and under*

Gorged larvae were exposed to the following combinations of humidity and low temperature:

Temperature ° C.	R.H.
2–3	100, 90 — — — —
10	100, 95, 90, 85 — —
14–16	100, 95, 90, 85, 80, 75

2–3° C. The larvae at 2–3° C., 100 R.H., were transferred after 100 days to 27·5° C., 95 R.H. (Those at 90 R.H. were found to be dead.) Moulting commenced on the 31st day.

10° C. The ticks at 10° C. were transferred to 27·5° C., 95 R.H., after 85 days. Moulting began as follows:

100 R.H., 27 days; 95 R.H., 24 days;
90 R.H., 27 days; 85 R.H., 27 days.

14–16° C. The larvae at this temperature were transferred to 27·5° C., 95 R.H., after 100 days, and moulted as follows:

R.H.	100	95	90
Shortest	Dead	24	24
Average	—	29	—

Tubes each containing six gorged nymphs were exposed to a temperature of 14–16° C., at R.H.s of 100, 95, 90 and 80, left for 100 days and then transferred to 27·5° C., 95 R.H.

Moulting occurred as follows:

R.H.	100	95	90	80
Shortest	Destroyed by accident	32	31	26
Average	—	34	32	31

Since the shortest and average periods for total development at 27·5° C., 95 R.H., are 24 and 30 days (larvae), and 31 and 39 days (nymphs), it follows from these results that, at temperatures of 14–16° C. or below, there is no appreciable development after exposures for periods of 85 and 100 days.

An unusual feature of all the results with low-temperature experiments was the high mortality among the ticks. Even at 20° C. the majority of the nymphs and larvae exposed for long periods died. It is not known whether this was due to changes in the gaseous contents of the closed systems during these prolonged periods, to changes in the moisture conditions as a result of absorption of CO_2 by the KOH solutions, or to the effect of exposure to an unchanging temperature unfavourable for rapid development.

(*d*) *Development at temperatures of over* 35° C.

Several attempts were made to induce both larvae and nymphs to develop at 37·5° C., various humidities being used, but in every case the ticks died. An effort was then made to find the rate of development at this temperature by removing the ticks before the lethal effect had time to operate, and allowing them to complete their development at a lower temperature. Three tubes of ten larvae each were set at 37·5° C. in saturated air; one tube was removed to 30° C. after 5 days, one after 10, and one after 15 days. The larvae in all three tubes died. It would appear, therefore, that the upper limit of temperature for development lies between 35 and 37·5° C., the latter temperature being definitely unfavourable even when ticks are exposed to it for only a few days.

From the foregoing experimental results, the developmental velocity curve of ticks can be produced above and below the medial temperature range. In Fig. 4, velocity curves of development of gorged larvae have been plotted

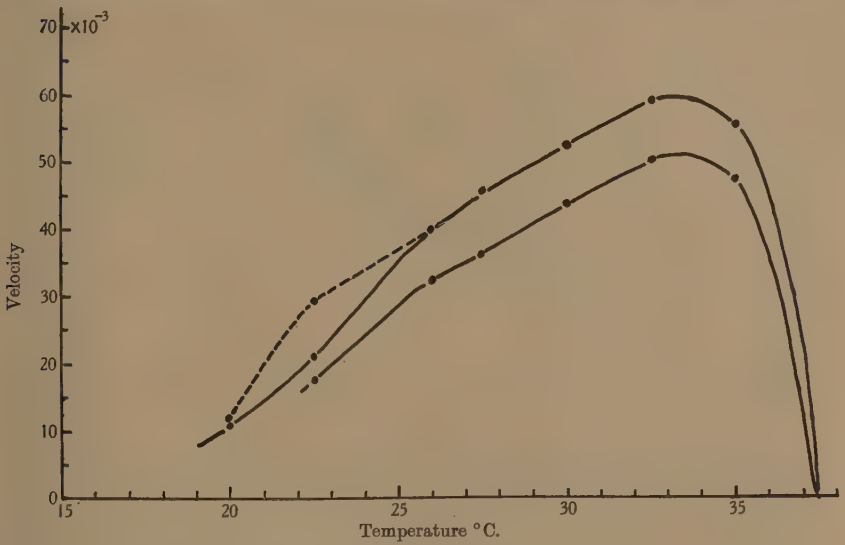

Fig. 4. The velocity curve of gorged larvae, based on the highest and average velocities obtained in saturated air. The broken line represents the highest velocity at optimum humidities.

against temperature, the curves being based on the shortest and average times taken in saturated air at different temperatures. It will be seen that, although within the limits of 26 and 32·5° C., the curve is approximately a straight line; above and below these limits the character of the curve alters. At temperatures over 32·5° C., the curve tends to fall away, at first slightly, and then very steeply to zero velocity at 37·5° C. Below 26° C. there would appear to be a slight relative retardation of development with decreasing temperatures, the threshold of development lying in the neighbourhood of 15° C.

An interesting point raised by the above findings is the question of the correct humidity to be considered when plotting full velocity curves. It has been shown above that the humidity conditions under which development proceeds most rapidly are not the same for all temperatures. Where the "optimum" humidity is below saturation, one may reasonably assume that conditions either more or less humid than the optimum exercise an inhibitory influence which retards the velocity rate. It would, therefore, appear to be more accurate to take the optimum humidity for each temperature, rather than a constant condition, *e.g.* saturation, when plotting a velocity curve representing the response of an organism to temperature alone. Otherwise, the curve is not a true reflection of the response evoked by temperature, since at certain temperatures the result obtained represents the response after modification by the retardation effect of unfavourable humidity.

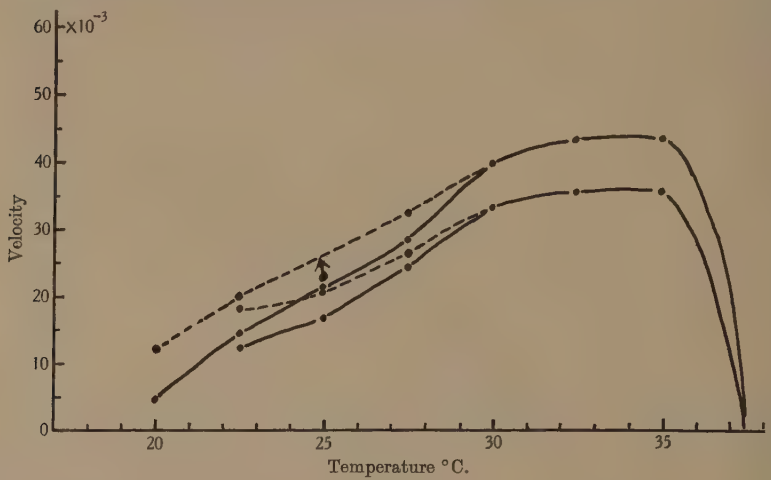

Fig. 5. The velocity curves of gorged nymphs. The whole lines indicate the highest and average velocities in saturated air; the dotted lines, the velocities under optimum humidity conditions.

In the case of larvae, the optimum humidity conditions coincide with saturation at temperatures down to 22·5–26° C.; the curve for highest velocity at any humidity will, therefore, coincide with that depicted in Fig. 4 for the greater part of its length. The aberration (dotted line) suggests that the true velocity curve retains its straight line character down to 22·5° C. before exhibiting deceleration. On the other hand, as shown in Table II, the optimum humidity for gorged nymphs departs from saturation conditions at higher temperatures. Fig. 5 depicts the velocity curves for gorged nymphs both under constant saturated air conditions and at optimum humidities. It is clear from this figure that the velocity curve under a constant humidity condition may be misleading; the curves for saturated air (unbroken lines) are

irregular, whereas those for optimum moisture conditions (broken lines) exhibit the conventional straight-line character for medial temperatures.

(*e*) *The threshold of development and the thermal-constant theory*

As has been pointed out above, when the reciprocals of the times taken for development are plotted against temperature, the resulting curve expresses the velocity of development. Where this curve transects the temperature axis the velocity is zero, and this temperature represents the theoretical threshold of development. Many workers have obtained this point by producing the reciprocal of the development curve at medial temperatures, on the assumption that the development curve is a hyperbola and its reciprocal, therefore, a straight line. It is now generally admitted, however, that the hyperbolic zero is only an approximate indication of the threshold of development.

If the development curve be admitted a hyperbola, the product of the ordinates of any point will be a constant. The ordinates in such a case are the time for development and the number of effective degrees, or degrees above the threshold of development. That is, development would require a fixed number of day-degrees, or the principle may be expressed by saying that exposure for x days at a temperature y degrees over the threshold has the same developmental effect on the organism as exposure for y days at a temperature x degrees over the threshold. This thermal-constant theory is much used to-day by insect ecologists, but it has the serious objection that it assumes the development curve to be a hyperbola, whereas the curves of insect development have been shown to be represented by hyperbolae only within limited temperature ranges. It is of interest to apply the theory to the development curve of the tick.

A development curve represented by a true hyperbola can be expressed by the formula $xy=c$, where y is the time for development, and x the number of effective degrees of temperature. The value x may be expressed as $T-K$, where K is the threshold of development, and T the recorded temperature. Since C, the thermal constant, is fixed, the value K may be obtained if the ordinates of two points on the curve are known. Thus, if $(T-K)\ Y=C$ and $(T_1-K)\ Y_1=C$, then

$$K=\frac{TY-T_1Y_1}{Y-Y_1}.$$

Taking the results given in Table I, we find that K has the following calculated values:

Temperatures °C.		K °C.
22·5	26	20
22·5	27·5	18
26	27·5	15
26	30	13
27·5	30	12
27·5	32·5	10
30	32·5	9

It follows, therefore, since K is not a constant value, that the full developmental velocity curve cannot be expressed as a hyperbola; that is, each day-degree has not a uniform developmental value.

It is apparent, therefore, that the thermal-constant theory cannot safely be used here as a basis for calculating the threshold of development.

IV. The temperature-velocity relation with varying temperatures

It has been shown that a day-degree has not a fixed developmental value, but varies with different temperatures. It now remains to be seen whether, the temperature being fixed, the application of a fixed number of day-degrees produces a constant developmental response, or whether the intensity of response is subject to variation according to the previous history of the organism. It has been shown that exposure to a temperature well below the threshold of development appears to produce a slight retardation in the response to a subsequent favourable temperature; there remains the possibility that exposure for a period to one temperature within the favourable limits may affect the velocity of development when the organism is later subjected to another favourable temperature.

Two tubes each containing ten fully gorged larvae were set at 25° and 35° C. respectively, each under optimum humidity conditions, *i.e.* saturation.

After exposure for 12 days, the tubes were interchanged and left until moulting was completed.

The results were as follows:

Table VI. *Larvae.*

	First temp. ° C.	Period exposed days	Second temp. ° C.	Shortest time	Average time
Ticks exposed to successively lower temperatures	25	12	35	10	12
Ticks exposed to successively higher temperatures	35	12	25	12	15

The velocity values for 25° and 35° C. when the ticks are exposed to a fixed temperature throughout their development may be obtained from the velocity curve (Fig. 4) by interpolation in the case of 25° C., and by direct reading for 35° C.

They are: 25° C. Highest, 1/27. Average, 1/33.
35° C. Highest, 1/18. Average, 1/21.

Let x = velocity at 25°, x_1 that at 35° C. Thus

$$\frac{x}{x_1} = \frac{18}{27} = \frac{2}{3} \quad \text{(highest);}$$

or

$$= \frac{21}{33} = \frac{7}{11} \quad \text{(average).}$$

Applying these ratios to the above results, we find that the calculated times for development at the second temperature in each case should be as follows:

Rising temperatures:

Development at 35° C. = 18 − 2/3 of 12 = 10 days at 35° C. (highest velocity);
or 21 − 7/11 of 12 = 13 days (average velocity).

Falling temperatures:

Development at 25° C. = 27 − 3/2 of 12 = 9 days at 25° C. (highest velocity);
or 33 − 11/7 of 12 = 14 days (average velocity).

These theoretical results compare with the observed results as follows:

Falling temperatures				Rising temperatures			
Observed		Calculated		Observed		Calculated	
12	15	9	14	10	12	10	13

There is no significant difference between the observed and calculated results.

It follows, therefore, that, humidity conditions being uniformly optimum, unit exposure to a fixed temperature has a fixed developmental effect, irrespective of the previous history of the organism in regard to temperature, except where this involves exposure to lethal high temperatures, or to very low temperatures.

This quantitative agreement between temperature and velocity is of interest in view of a recent paper by Falke (1931). This author stated that development of *Ixodes ricinus* underwent in the winter months an interruption which could not be altered by the employment of favourable conditions. He suggested that development may be divided into two phases, the *Vorruhestadium*, which lasts for 2 or 3 days, and during which the ticks are active, and the *Ruhestadium*, or stage of inactivity. Ticks which have not reached the *Ruhestadium*, or resting stage, at the onset of winter conditions cannot do so until the spring, and thus overwintering of gorged ticks occurs. Once the resting state is reached, the rate of development exhibits no appreciable variation, development occupying approximately 25 days.

Were this the case, exposure at different temperatures during the resting stage would not affect the velocity of development, provided the ticks were exposed to an initial fixed temperature during their pre-resting stage. That is, ticks exposed to an initial temperature of, say, 25° C., would complete development in a fixed time irrespective of whether they were removed to another temperature a few days later, or left at 25° C. The experimental results described above show that this is not the case. Further, since many of the experiments described in this paper were carried out in winter, it follows that, in this country at any rate, there is no seasonal diapause, such as Falke suggests, in the physiological activity of the tick.

V. Intereffects of temperature and humidity variations

The previous section dealt with the effects of temperature and humidity individually, variations in one factor being considered under conditions where the other factor was constant. In nature, however, both factors operate as variables simultaneously; their intereffects and the effect of their simultaneous variations must, therefore, be considered.

It can be shown theoretically, from an analysis of the data obtained under constant conditions, that each factor intimately affects the potentiality of the other. If we consider the moisture conditions in the different experiments in terms of vapour pressure, we obtain a common vehicle of expression for both the temperature and the humidity factor. It is known that, temperature being constant, saturation deficiency varies inversely with the relative humidity, and can be calculated if the latter is known; humidity being constant, saturation deficiency varies directly with the temperature, and can be

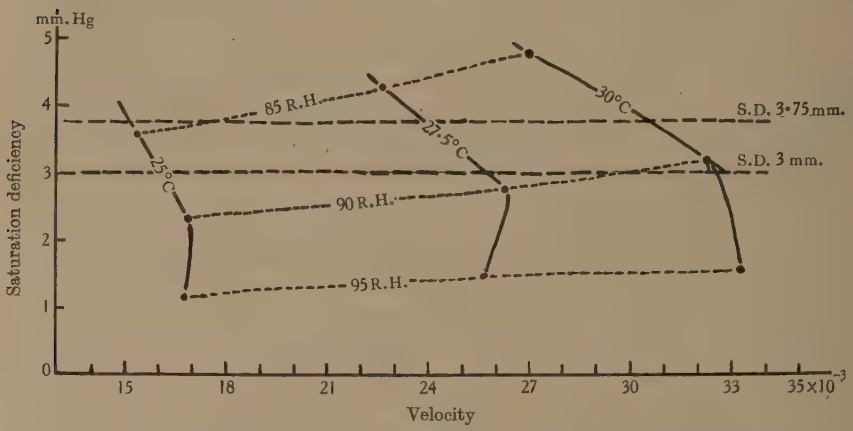

Fig. 6. Intereffects of temperature and humidity. The dotted lines represent the effect on developmental velocity of temperature variations at different humidities; the whole lines, the effect on velocity of relative humidity variations at different temperatures. The parts of the curves enclosed by the interrupted lines indicate the comparative velocity response at different temperatures to an absolute humidity increment (3·75 to 3 mm. deficiency).

calculated if the latter is known. It follows that, since saturation deficiency is a function of both relative humidity and of temperature, if developmental velocity be plotted against saturation deficiency, the resulting curves will illustrate the influence of temperature on the humidity effect, and the influence of humidity on the temperature effect.

In Fig. 6 the average velocity of development of gorged nymphs has been plotted against saturation deficiency, and the resulting curves illustrate the intereffects of the two factors. The dotted lines represent the effect on velocity of temperature variations at different relative humidities; the whole lines, the

effect on velocity of relative humidity variations at different temperatures, as follows:

(*a*) *The effect of a particular humidity change at different temperatures*

It will be seen that at all three temperatures there is an increasing velocity with decreasing saturation deficiency, *i.e.* with increasing humidity, up to a point, after which the influence of humidity on temperature response becomes less marked or even reversed. This principle has already been encountered (Fig. 3). If those limits of saturation deficiency be considered within which the responses at all temperatures are qualitatively similar, *i.e.* saturation deficiencies of 3–3·75 mm., the curves demonstrate a quantitative agreement between saturation deficiency and temperature response. Thus a decrease of absolute humidity from 3 to 3·75 mm. deficiency in the vapour pressure is equivalent to the following velocity retardations at different temperatures:

25° C., velocity retarded by	10/161	or 6·2 per cent.
27·5° C., „ „	18/258	or 7 per cent.
30° C., „ „	19/323 to 25/329	or 5·9–7·9 per cent.

There is no evidence here of a significant variation in the percentage reduction of velocity at the different temperatures; that is, at saturation deficiencies of 3 mm. or over, removal of a definite quantity of moisture from the air apparently retards development by an approximately fixed percentage, irrespective of temperature, *i.e.* irrespective of the total amount of moisture in the air.

Since a fixed quantity of moisture at different temperatures represents different proportions of the total amount present, it follows that a fixed change in velocity is produced at different temperatures by different degrees of change in the relative humidity. The converse of this is illustrated in the diagram, where a fixed change in degree of humidity produces different velocity retardation effects at different temperatures, *e.g.* R.H. reduced from 90 to 85 retards velocity:

At 25° C.	by 15/169	or 9 per cent.
At 27·5° C.	„ 36/263	„ 13·7 „
At 30° C.	„ 53/323	„ 16·4 „

It would appear, therefore, that there is a differential response to relative humidity variation at different temperatures, a greater response being evoked by the same percentage variation of humidity at high temperatures than at low. This does not hold in saturated air.

(*b*) *The effect of a particular temperature change at different humidities*

A decrease of temperature from 30° to 25° C. retards velocity by the following percentages at different humidity conditions:

Saturation deficiency	R.H.	Percentage retardation
Between 1 and 2 mm.	About 95	50
„ 2 „ 3 „	„ 90	47
„ 3 „ 4 „	„ 85	43

The regular trend of these results suggests that there may be a differential response to temperature variation at different conditions of humidity, the response being greater at higher humidities. This would appear to hold good up to complete saturation of the air.

(c) *Simultaneous variations in temperature and humidity*

The effects of different static conditions of one factor on the responses evoked by variations in the other have been discussed. The intereffects of the simultaneous operation of variations in both factors remain to be considered, and have been subjected to experimental analysis.

A number of tubes each containing ten newly gorged larvae were exposed to different combinations of temperature and humidity, as follows: One series of three tubes was exposed to progressively higher temperatures and humidities for different periods, and the time taken to moult under the last set of conditions observed. The second series was exposed to progressively lower temperatures and humidities, and the times taken to moult observed.

The experiment and results may be tabulated as follows:

Table VII. *Larvae.*

					35° C., 100 R.H.	
Series 1:	Tube	25° C., 90 R.H.		30° C., 95 R.H.	Shortest	Average
ascending temperatures	1	10 days		10 days	10 days	15 days
	2	15 „		5 „	13 „	14 „
	3	5 „		15 „	9 „	? „
Series 2:		Shortest	Average			
descending temperatures	1	2 days	6 days	10 days	10 days	
	2	0 „	3 „	5 „	15 „	
	3	8 „	9 „	15 „	5 „	

These observed results were compared with the results calculated from the data in Table I (the value for 25° C., 90 R.H., being obtained by interpolation).

The control values are:

35° C., 100 R.H. Shortest, 18. Average, 21.
30° C., 95 „ „ 22. „ 25.
25° C., 90 „ „ 32. „ 43.

Tube 1 on the ascending scale may be taken to exemplify the method of calculation.

Let x = shortest number of days at 35° C., and let complete development = unity.

Thus
$$\frac{10}{32}+\frac{10}{22}+\frac{x}{18}=1;$$
$$\frac{x}{18}=1-\frac{10}{32}-\frac{10}{22}=\frac{41}{176};$$
i.e.
$$x=4.$$

Similarly, if x = average number of days at 35° C.,

$$\frac{10}{43}+\frac{10}{25}+\frac{x}{21}=1,$$

or $$x=7\text{–}8 \text{ days.}$$

Table VIII compares the observed and calculated results for the two series.

Table VIII

			35° C., 100 R.H.			
			Observed		Calculated	
Series 1	25° C., 90 R.H.	30° C., 95 R.H.				
Tube 1	10	10	10	15	4	7–8
„ 2	15	5	13	14	5–6	9–10
„ 3	5	15	9	?	2–3	6
			25° C., 90 R.H.			
			Observed		Calculated	
Series 2	35° C., 100 R.H.	30° C., 95 R.H.				
Tube 1	10	10	2–3	7	0	5
„ 2	15	5	0	3	0	3–4
„ 3	5	15	8	9	1	6–7

It will be seen that, in comparison with the temperature-velocity relation under optimum-humidity conditions (Table VI), there is here an appreciable discrepancy between the observed and calculated results. When the organism is exposed to different temperatures, and at the same time subjected to adverse humidity conditions of varying intensity, there is an apparent reduction in the degree of response to temperature increase.

Another experiment was set up, in which the moisture conditions were more severely unfavourable. A tube of twenty newly gorged larvae was exposed to 25° C., 85 R.H. After 6 days it was transferred to 30° C., 100 R.H. The first moult occurred on the 16th day of exposure to the last combination. Owing to a subsequent high mortality from mould, it was not possible to determine the average time taken. If, however, the shortest time at 35° C., 100 R.H., is calculated, from the equation $\frac{6}{41}+\frac{8}{23}+\frac{x}{18}=1$, it will be seen that x, the theoretical time required at 35° C., is 9 days. The actual shortest time recorded was 16 days. This supports the results of the previous experiment.

There is a suggestion also, from the results given in Table VIII, Series 2, although the figures are not so striking as those in Series 1, that a reduction of temperature under unfavourable moisture conditions imposes a severer check on development than would theoretically be expected. Except in Tube 3, however, the differences are too slight to be regarded as significant.

Since it has been shown (Section IV) that, under constantly favourable humidity conditions, temperature has a uniform quantitative effect on development, it follows that the apparent differences in the quantitative effect of particular temperatures in these experiments are due to the inhibitory effect of adverse humidity conditions. Whatever be the correct interpretation of this inhibitory effect, it is evident that humidity has an intimate controlling

influence on the responsiveness of the organism to temperature variations; it would also appear that the intensity of operation of this retarding influence varies with the degree of departure of these factors from the optimum, the relation being direct in the case of humidity, and inverse in the case of temperature. Thus, the discrepancy is more marked in Series 1 than in Series 2: the ticks in this series were exposed for relatively longer periods to high, *i.e.* optimum, temperatures than those in Series 2. Further, in Series 1, where the discrepancies are well marked, it will be seen that they are more pronounced in the case of the ticks exposed for 10 and 15 days to a relatively low humidity than in the case of those exposed to this humidity for only 5 days.

VI. Influence of barometric pressure on development and ecdysis

(*a*) *Influence of pressure on development*

The available evidence on the influence of barometric pressure on development of arthropods is, as is indeed the case with other climatic factors, all in relation to insects. The evidence suggests that insects are capable of tolerating wider variations of pressure conditions than would normally occur in nature. The rate of development also of insects is apparently insusceptible of alteration in response to atmospheric pressure variations, even of a high order (Uvarov, 1931), although some workers have obtained a quickening of development under constant conditions of reduced pressure. In view of these findings, the following unexpected results with gorged ticks are of interest.

Gorged larvae when a few days old were exposed at 30° C. in saturated air to pressures of 10 and 5 lb. over normal, to normal atmospheric pressure, and to 5 and 8 lb. under normal, one tube of ten larvae being exposed to each condition.

The pressure conditions were obtained as follows: The stopper of each closed humidity system was pierced with two pieces of glass tubing, over which were fitted pieces of rubber tubing. Reduced pressures were obtained by aspiration from one tube, the pressure being recorded on a vacuum gauge inserted into the other. Increased pressures were obtained by means of an ordinary pump, fitted with a valve, the pressure being recorded on a pressure gauge. The tubes were then tightly clamped, and the stoppers, which were tied down securely, were sealed with paraffin wax.

Moulting commenced in the control tube at normal pressure (N) on the 18th day. Two days later the experimental systems were opened, and the larvae examined for moulting.

Pressure $N + 10$ lb.	10 moults
„ $N + 5$ lb.	7 „
„ N	8 „
„ $N - 5$ lb.	Nil
„ $N - 8$ lb.	Nil

The experiment was repeated, larvae gorged 4 days previously being set, twenty to a tube, at pressures of $N+$(10–12 lb.), $N+$(6–7 lb.), N, $N-5$ lb. and $N-$(7–8 lb.).

The first moult occurred in the controls on the 18th day, whereupon the other systems were examined.

Pressure	$N+$10–12 lb.	All 20 had moulted
„	$N+$ 6–7 lb.	19 moults
„	N	1 moult
„	$N-$ 5 lb.	Nil
„	$N-$ 7–8 lb.	Nil

The reduced pressure systems, which had not been opened, were left for another fortnight, during which no moulting occurred. The ticks in these systems were then removed to normal pressure at 27·5° C., 100 R.H. On examination a few days later, they were found to be dead.

It appears, therefore, that in the case of *Ixodes ricinus* development can be accelerated by increased pressure, and inhibited, or at least markedly retarded, by reduced pressures, the ticks actually being killed by prolonged exposures to pressures of 5 lb. or more under normal.

The experimental pressure variations used, however, were of a much greater order than would normally occur in nature, and there is no evidence for supposing that slight fluctuations, such as occur naturally, produce appreciable effects on development.

(*b*) *Influence of pressure on ecdysis*

Pictet (cited by Uvarov, 1931) concluded from his work on butterfly pupae that emergence took place as a result of a fall in barometric pressure altering the ratio between the pressure of the body fluids and that of the outer air. The wide limits of variation for individual moulting of ticks kept under identical conditions, when these were static (Tables I and II), suggested that this hypothesis might be applicable to ticks. The results of the following experiments, however, lend no support to this view.

Sixty larvae were kept at 30° C., 100 R.H., until moulting had commenced. They were then removed and forty of the remaining unmoulted larvae, presumably near the point of hatching, were divided into four lots of ten, and exposed to (1) a pressure of $N+5$ lb., (2) a pressure of $N-3$ lb., (3) a pressure which fluctuated between these two limits, and (4) normal pressure.

The tubes were examined for each of the following 5 days, without the systems being opened. By the 5th day moulting was completed among those set at increased, normal and fluctuating pressures, no appreciable difference being observed in the rates of moulting. In the tube exposed to reduced pressure, no moulting occurred by the 2nd day, when the system accidentally broke down.

The experiment was repeated; five tubes of twenty larvae each were kept at 30° C., 100 R.H., until moulting commenced. The tubes on examination were found to contain nineteen, nineteen, nineteen, eighteen and fifteen

unmoulted ticks respectively. The last tube was discarded, and the other four set at (1) $N+7$ lb., (2) $N-5$ lb., (3) normal, and (4) a pressure fluctuating daily from $N+7$ to $N-5$ lb., commencing with $N+7$ lb. The result was as follows:

Pressure	3rd day	7th day
Control	6 moults	11 moults
Positive pressure	8 „	17 „
Negative pressure	3 „	6 „
Fluctuating pressure	7 „	Discontinued

It will be seen that, if anything, a retardation of ecdysis results from a reduction of barometric pressure. The pressure reduction in this case, however, is greater than would occur in nature, and it is probable that, as regards the tick, normal barometric variations exercise no acceleration or inhibition effect on ecdysis.

VII. Discussion

From a review of the foregoing experimental results, some conceptions of the relation of the developing tick to the main climatic factors affecting it may be formulated. It is clear that the two outstanding factors influencing development are temperature and moisture, and these appear to be important both in their separate and their combined effects.

An interesting feature of the results is the wide range of temperature over which development, at a greater or lesser rate, is possible. From a lower limit in the region of 15° C. to an upper limit of 35° C., development is appreciable, although the velocity varies greatly between these extremes. Even more striking is the comparatively small range of humidity conditions within which survival and development are possible, the tick in this respect differing markedly from the majority of insects. Its lower favourable limit is 80–85 per cent. saturation, while, for the greater part of the temperature range favourable for comparatively rapid development, the optimum moisture condition is saturation vapour pressure.

In addition to this direct limiting effect, humidity also exercises a profound influence on the response of the tick to temperature. Under static conditions of both factors, the velocity of development is slowed down by suboptimal humidity conditions. Further, the quantitative response of the organism to temperature variation is affected by variations of the humidity factor.

It is clear from a consideration of the mortality percentages at different temperatures that the optimum temperature for development is not simply that temperature at which development proceeds most rapidly. Pearls (1927) defines the optimum for insects as that temperature range within which the relatively greatest percentage of individuals accomplish their development within the relatively shortest period. Before the ideal optimum could be decided, the comparative value to the race of unit time-shortening and of the individual would need to be known, for it is conceivable that percentage mortality, up to a certain point, is sufficiently compensated by the decrease in the time spent in the inactive, non-parasitic phase.

Theoretically, the greatest efficiency would be found at that temperature

at which the highest velocity was reached under optimum humidity conditions, provided all survived, *i.e.* optimum would equal 100 per cent. survival, multiplied by the highest velocity. The actual efficiency at this temperature would, therefore, be the product of the percentage survival and the velocity, the whole expressed as a percentage of the theoretical optimum. Similarly, the efficiency at any temperature might be calculated. Thus, in the case of gorged larvae, the theoretical optimum is 32·5° C., at which temperature the average velocity is 1/20. The theoretical optimum efficiency, therefore, is $100 \times 1/20$ or 5.

The actual moulting efficiency for larvae

$$\text{at } 32{\cdot}5^\circ \text{ C.} = \frac{\text{percentage survival} \times \text{velocity} \times 100}{\text{theoretical optimum efficiency}} = 100 \text{ per cent.};$$

$$\text{at } 35^\circ \text{ C.} = 65 \times \frac{1}{21} \times 100 \times \frac{1}{5} = 62 \text{ per cent.};$$

$$\text{at } 30^\circ \text{ C.} = 100 \times \frac{1}{23} \times 100 \times \frac{1}{5} = 87 \text{ per cent.}$$

Thus, if we compare the product of the percentage survival and the velocity at each temperature with a theoretical optimum efficiency, we can obtain a relative scale of values for the "moulting efficiency" at different temperatures. In the case of larvae it would appear from the results in Table I that the efficiency curve exhibits a sharp peak at the optimum temperature of 32·5° C., but it is very doubtful if this result would be maintained over a wider series of experiments. More probably an increasing percentage mortality would accompany increasing temperatures, especially at 30° C. and over, since in nature such temperatures are not usually attained in moist soil—the normal habitat of the gorged tick. In Table II also the results are based on too small numbers, and, consequently, the mortality values are too irregular to allow of an accurate interpretation of the relative "moulting efficiency" of different temperatures.

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A CASE OF MYIASIS, DUE TO *CALLIPHORA ERYTHROCEPHALA*, OCCURRING IN MAN

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The case described in this note is believed to be unique, for no similar record has been found after search of the relevant literature. The subject of myiasis has been carefully reviewed and abstracted by Huber (1899), Umaña (1915) and Bedford (1927), and no cogent references of more recent date have been discovered.

The patient, a man aged 59 years, was employed as a farm labourer in the local fenland district.

Previous medical history. In August, 1929, the patient was operated upon for extravasation of urine, following obstinate urethral strictures. Suprapubic drainage was necessary until his death. In December, 1931, a second operation for stones was required, and, during the following year, a third operation was performed for a perineal fistula. From that date until a short time before his death nothing of moment occurred. He was ambulatory, but was unable to follow his usual vocation.

Recent medical history. Early in October, 1933, a sudden attack of vomiting occurred. The vomites were reported to have contained living maggots. A second and more severe bout of vomiting happened, on October 14th, when medical advice was sought. Upon this occasion about a pint and a half of blood-laden material was vomited. The vomites contained living maggots—others were found in the faeces and in the discharge from the bladder. The bladder was found to be in a septic and consequently malodorous condition. A few days before death the maggots disappeared from the vomites, but they persisted in the faeces and in the bladder. The original specimens, received for examination, came from the last two sources, but unfortunately most of the maggots were dead, presumably having been drowned in the liquid specimens. By request, further specimens were collected in as dry a condition as possible and were transmitted without delay to the laboratory. Upon arrival all the maggots that were found alive were set aside so that they might undergo pupation. In all 114 flies were obtained. These were identified as follows:

Calliphora erythrocephala: 53♂, 58♀.
C. vomitoria: 1♂, 1♀.
Lucilia (? *sericola*): 1♂.

The patient died on November 2nd, 1933—approximately one month after the first maggots were observed. In the interval between the two attacks of vomiting, the patient partook of fried sausages. There was no reason to suspect

them, for the maggots had been observed before the sausages were eaten. Apart from the sausages no history of possibly contaminated food having been consumed was obtainable. Just before death, the patient was in an extremely collapsed condition and almost pulseless for some hours (about a day). Permission for a post-mortem examination was refused.

Comments. The exact duration of the infestation is unknown. The dominant larvae, which pupated, were those of *Calliphora erythrocephala.*

In the absence of post-mortem confirmation, no definite conclusions may be drawn. It is probable, however, that some vesico-intestinal communication had been established. This would account for the voiding of the larvae in the faeces, in the washings from the bladder, and possibly also for their presence in the vomites. In the last-named instance, however, it must be borne in mind that eggs, deposited in the unhealthy suprapubic sinus by flies attracted to the part through the evil smell, may have been conveyed to the patient's mouth by his own fingers and then swallowed—having subsequently hatched they were then expelled as maggots with the vomit.

The original site of the infestation was probably the permanent opening into the bladder made at the first operation.

I am greatly indebted to Dr W. M. Wilson, of Littleport, for his permission to publish this case and for the clinical notes he supplied, also to Prof. D. Keilin, F.R.S., for his advice and for the identification of the flies arising from the larvae.

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